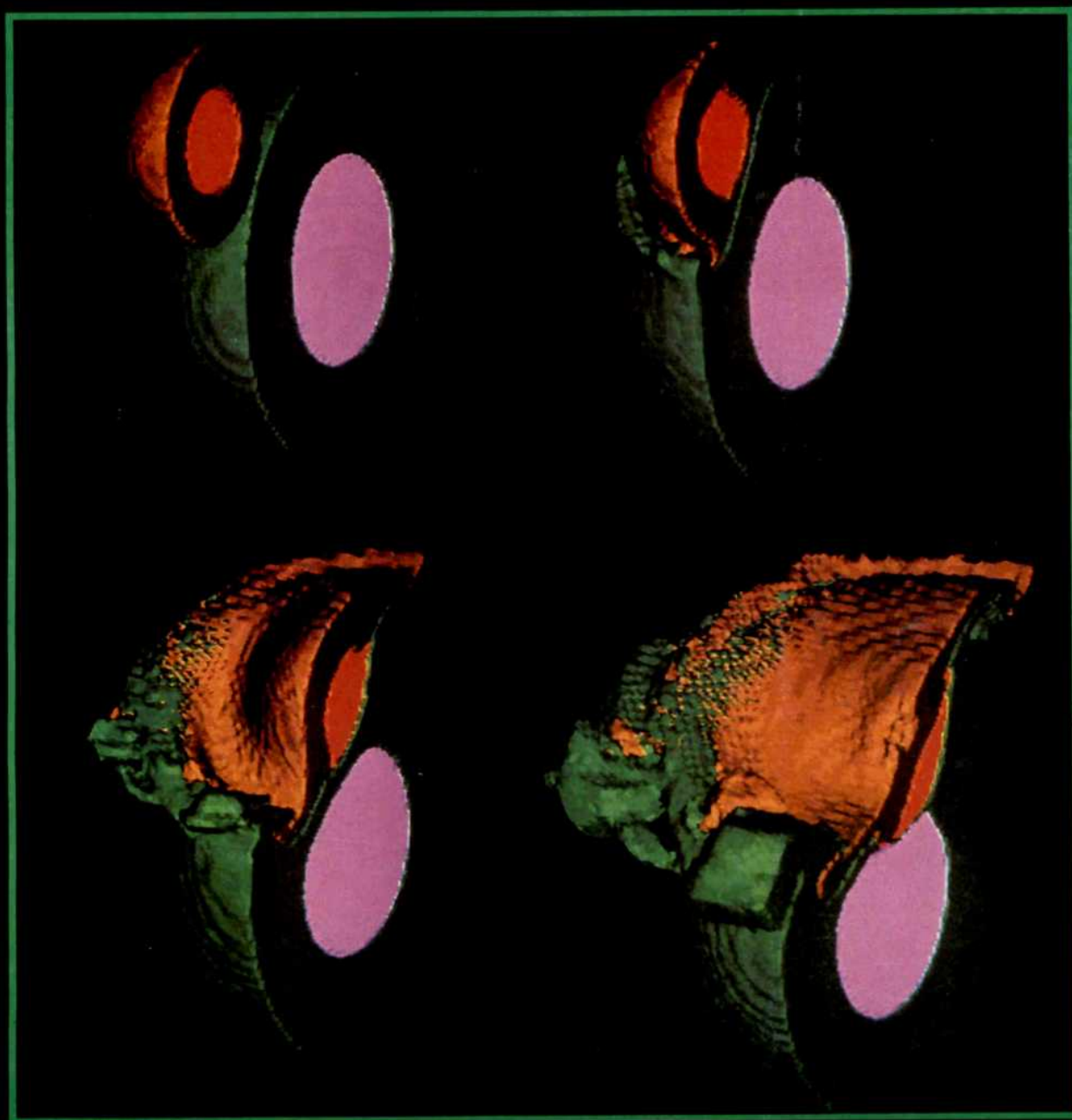


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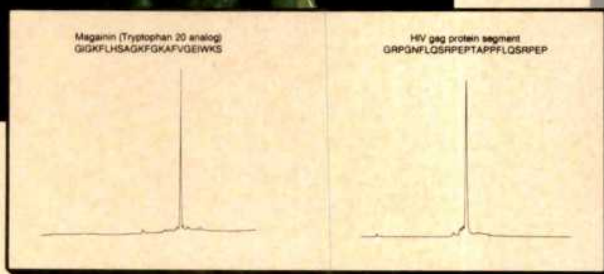
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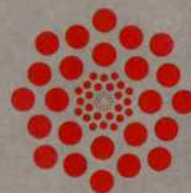
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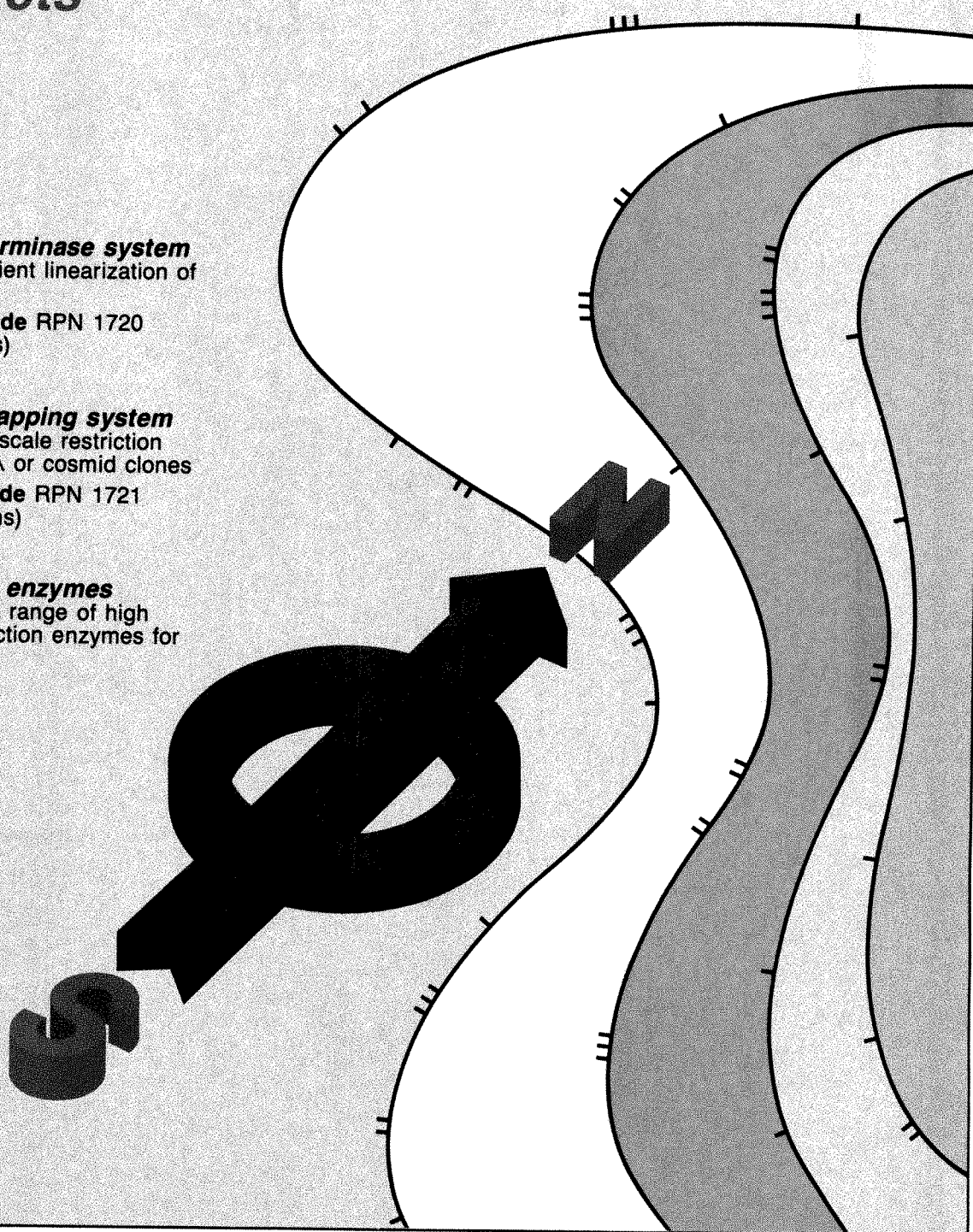
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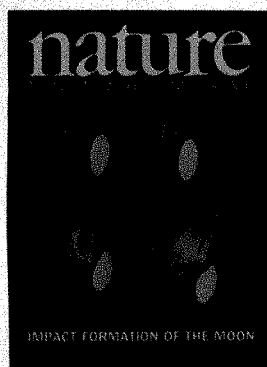


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2 March 1989

Vol. 338 Issue no. 6210

The unusual orbital characteristics of the Earth-Moon system can be explained if the Moon formed from the debris of a collision between the early Earth and a Mars-sized body. The cover shows four stages in a computer simulation of the collision. See pages 29 and 19.

## THIS WEEK

### Last fall's drought

The 'greenhouse effect' was thought by some to be responsible for last year's US drought. But a complex global weather-prediction model links the drought to sea-surface temperature anomalies — one factor being the arrival of exceptionally cold water in the tropical Pacific. Pages 54 and 15. Congress takes steps, page 3

### Diabetes insight

The cloning of the gene for the insulin-regulated glucose transporter of adipose and muscle tissue could lead to new insights into non-insulin-dependent diabetes mellitus, page 83.

### Out of order

Muon spin rotation studies of a recently discovered electron-doped copper oxide superconductor reveal that the material's static magnetic order decreases with temperature and is totally destroyed when it becomes superconducting — as in hole-doped superconductors, page 49. Patents scramble, page 5

### Retinal decay gene

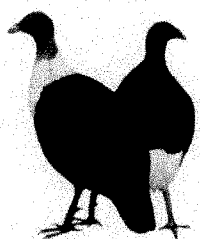
The characterization of a gene encoding a form of hereditary retinal degeneration in the mouse may make it possible to identify the cause of some cases of hereditary retinal degeneration in humans, page 70.

### Yeast recombination

Genetic studies in the yeast *Saccharomyces cerevisiae* have led to the identification of a chromosomal site for the initiation of homologous recombination. Both DNA strands at this site are cut during meiosis, supporting one current model for the mechanism of homologous recombination. Pages 35, 87 and 14.

## Political birds

The white-breasted guineafowl is but one of Africa's rare birds. Its extinction may be hastened by



Martin Woodcock

the exclusion of South Africans from the Pan-African Ornithological Congress. Commentary by T. M. Crowe, page 11.

## Fuelling the galaxies

Active galactic nuclei are usually thought to be powered by accretion onto a supermassive black hole. A mechanism for fuelling the nuclei, which relies on a 'stellar bar' instability sweeping the host galaxy's interstellar medium inwards, is described on page 45.

## One and the same

A *Drosophila* gene originally recognized by the effect of its mutation on visual transduction encodes a product closely related in sequence to cyclophilin, a mammalian protein that binds the immunosuppressive drug cyclosporin A. This is particularly intriguing in the light of the recent discovery that cyclophilin is identical to prolyl isomerase, an enzyme that facilitates protein folding. Page 67.

## Uranium frenzy

A compelling tale of the modern American West is the subject of a book review by Daniel J. Kevles, page 25.

## Guide to Authors

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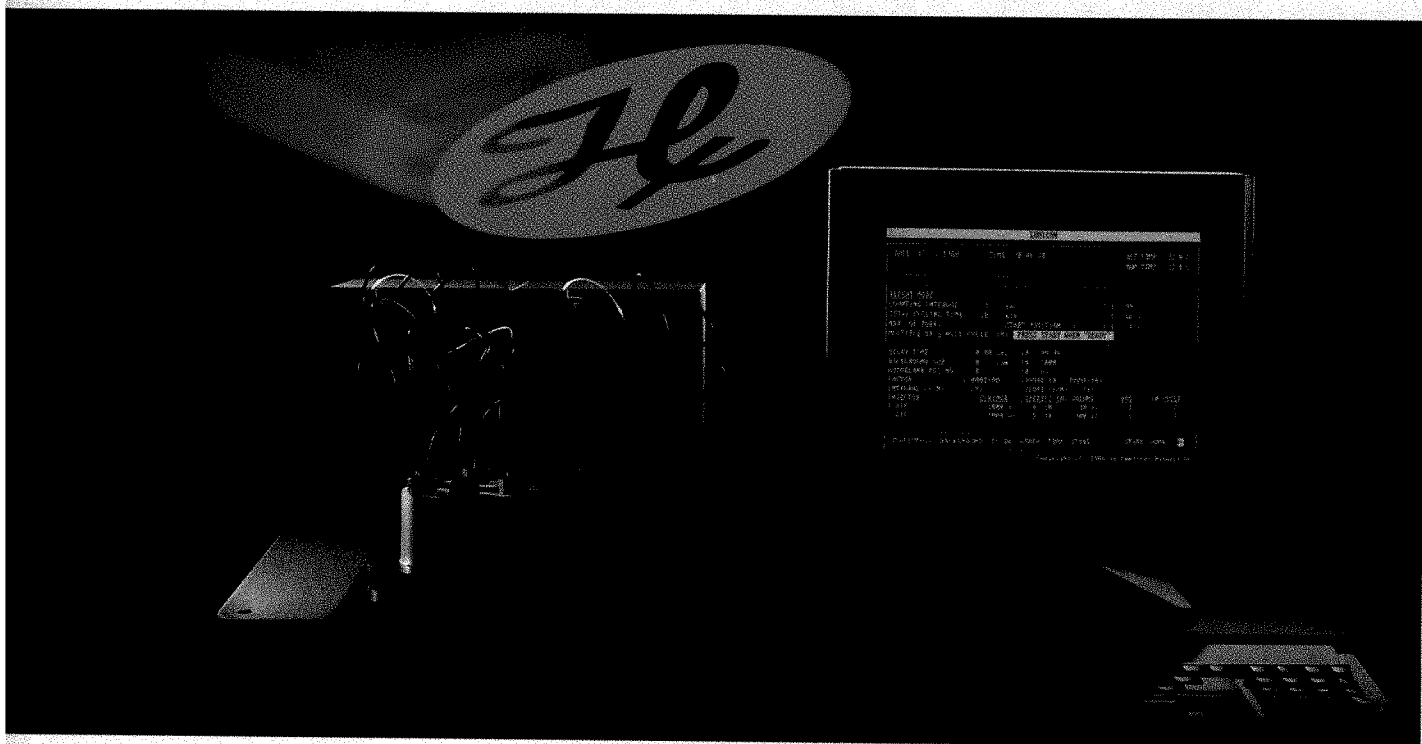
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Geochemical implications of the formation of the Moon by a single giant impact

H E Newsom & S R Taylor

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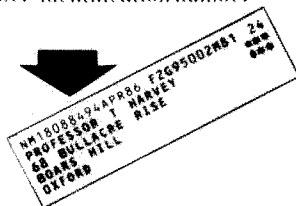
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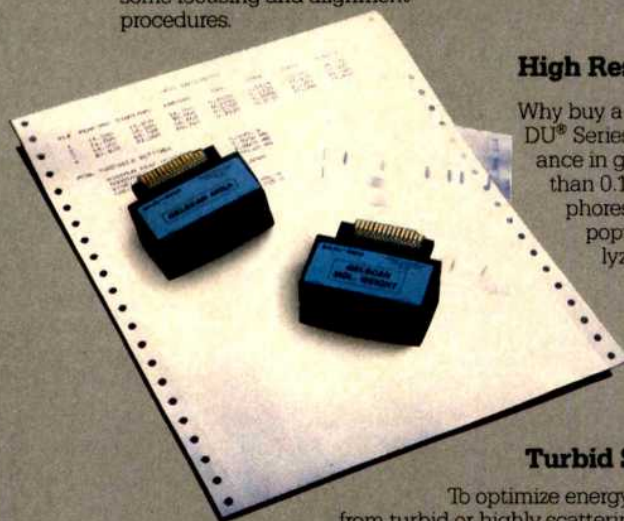
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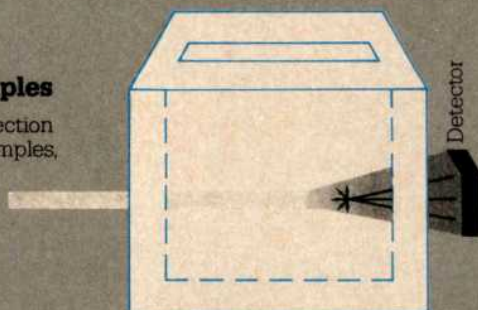


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Rapid isolation of RNA by a guanidinium thiocyanate/cesium chloride gradient method: T.H. Turpen, O.M. Griffith, Bio Techniques 4, 11 (1986).

RNA extraction by the guanidine thiocyanate procedure: R. McGookin, Meth. Mo. Biol. 2, 113 (1984).

Potent protein denaturant used, e.g. in the isolation of intact ribonuclease acid in the presence of ribonuclease.

J.M. Chirgwin et al., Biochemistry 18, 5294 (1979); S.A. Cockie et al., J. Biol. Chem. 253, 8019 (1978); W.N. Pollon, J. F. Bertes, ibid. 254, 3462 (1979); R. McCandless et al., Methods Enzymol. 79, 51 (1981); P.M. Lizardi, ibid. 96, 24 (1983).

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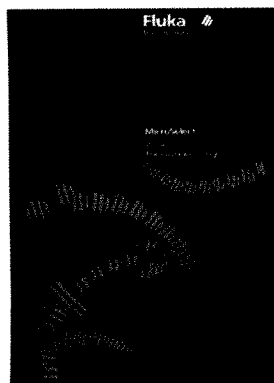
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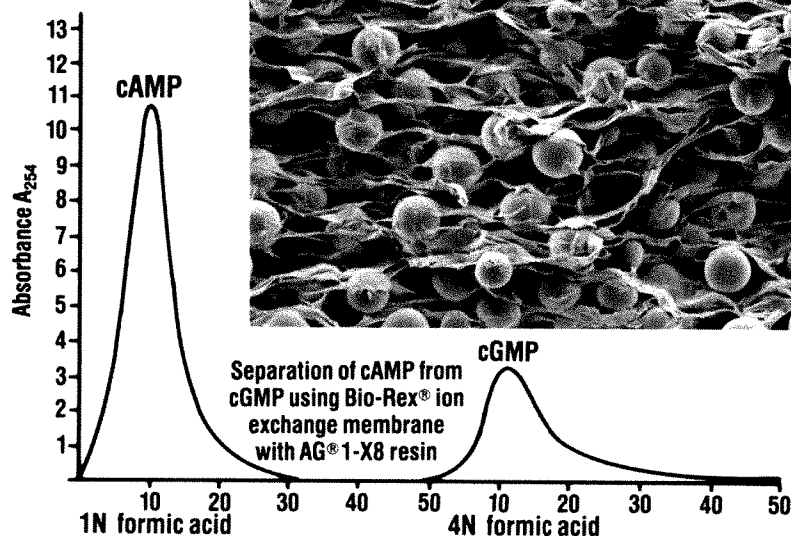
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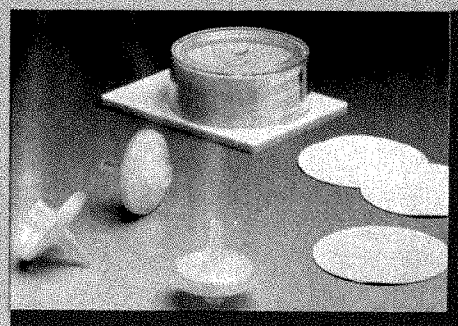
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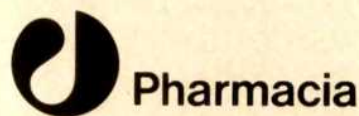
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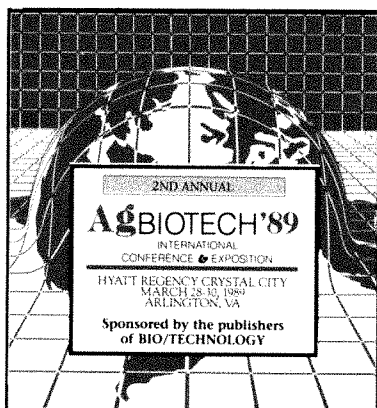
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## PROGRAM

### BUSINESS FORUM

8:00 a.m. – 11:00 a.m.

Management Strategies  
 Market Sizes and Structures  
 Long Range Strategic Planning

Chair: David Wheat  
 Panelists: A. Barnes, G. Kidd, R. Laster

11:00 a.m. – 2:00 p.m.:

2:00 p.m. – 5:00 p.m.

Management Strategies  
 International Strategic Alliances  
 High Level Staffing

Chair: Kelly Kincannon  
 Panelists: C. Baker, J. Bouckaert, J. Marcus

Tuesday, March 28

8:00 a.m. – 11:00 a.m.

Regulation: Case Studies; Testing for Registering Microbial Pesticides

Chair: Edgar R. Butts and Robert B. Nicholas  
 Panelists: D. Glass, C. Hutchinson, R. Kahn, J. Panetta, F. Serdy, J. Swigert, S. Woodhead

11:00 a.m. – 2:00 p.m.:

2:00 p.m. – 5:00 p.m.

Commercial Financing

Chair: G. Steven Burrill  
 Panelists: C. Earl, R. Moshe, L. Stern, D. Wagster

Wednesday, March 29

8:00 a.m. – 11:00 a.m.

Patents: Animal; Plant

Chair: Kathleen Merrigan  
 Panelists: D. Beier, A. Douglas, J. Doyle, H. Lyman, L. McKenzie, K. O'Conner, R. Quisenberry

11:00 a.m. – 2:00 p.m.:

1:30 p.m. – 4:30 p.m.

Government Research Funding Policies

Chair: Gary B. Ellis  
 Panelists: R. Dull, V. Giddings, K. Hanna, W. Marshall, M. Phillips, J. Tavares

Thursday, March 30

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## AT A GLANCE

### SCIENCE AND TECHNICAL FORUM

Plant Molecular Biology	Animals	Tuesday, March 28
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.	
The Production of Transgenic Crops Dicots – Cotton, Soybean, Oil Seed Rape, Walnut Pine  Chair: Harvey Bialy Panelists: K. Barton, A. Dandekar, J. Fillatti, M. Hinchee	Molecular Strategies for Production Animal Improvement: Transgenes, Genome Mapping  Chair: Charles Arntzen	
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS		
2:00 p.m. – 5:00 p.m.	2:00 p.m. – 5:00 p.m.	Wednesday, March 29
The Production of Transgenic Crops Monocots – Rice, Maize, Asparagus  Chair: Indra Vasil Panelists: R. Shillito, M. Von Montagu, R. Wu	Molecular Strategies for Production Animal Improvement: Transgenes, Genome Mapping (continued)  Chair: Charles Arntzen	
Plant Molecular Biology	Animals	
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.	
Biotechnology, Plant Breeding and Crop Improvement  Chair: Marc Von Montagu Panelists: H. Donner, T. Helentjaris, L. Privalle, I. Vasil	Animal Healthcare Vaccines Diagnostics Immune Stimulation  Chair: Fred Brown Panelists: G. Ada, J. Cantrell, J. McMillen	Thursday, March 30
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS		
2:00 p.m. – 5:00 p.m.	2:00 p.m. – 5:00 p.m.	
Controlling Gene Expression  Chair: Harvey Bialy Panelists: L. Comai, R. Fuchs, C. Lamb	Animal Healthcare (continued)  Chair: Fred Brown Panelists: G. Ada, J. Cantrell, J. McMillen	
Perspectives	Biopesticides	Friday, March 31
8:00 a.m. – 11:00 a.m.	8:00 a.m. – 11:00 a.m.	
Nitrogen Fixation  Chair: Ethan Signer Panelists: D. Kahn, D.P. Verma, G. Walker	Biopesticides Bacillus thuringiensis Endophytes Baculovirus  Chair: Jerry Caulder	
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS		
1:30 p.m. – 4:30 p.m.	1:30 p.m. – 4:30 p.m.	Saturday, April 1
The International Picture  Chair: Mark Ratner Panelists: J. Cohen, P. Oram, W. Roca, M. Sondahl, G. Toenniessen	Biopesticides (continued)  Chair: Jerry Caulder	

### AgBIOTECH '89 Faculty Continued:

Larry McKenzie, American Farm Bureau  
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\*Robert B. Nicholas, McDermott, Will &  
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\*David Wheat, The Boston Capital Group  
Suzan H. Woodhead, Ricerca, Inc.  
Ray Wu, Cornell University  
  
\*Chairman  
Program Panelists listed as of 1/31/89.

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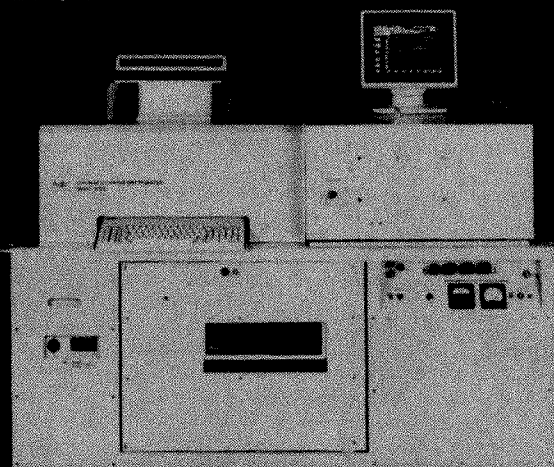
Tuesday, March 28 —  
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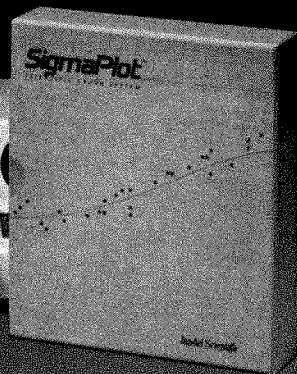
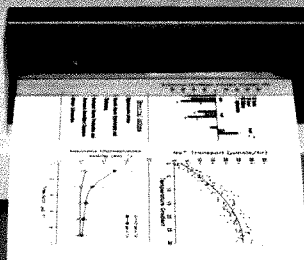
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Vol. 338 No. 6210 2 March 1989

## Bush badly needs good advice

President George Bush has promised to create better machinery for gathering technical advice, but time is passing. A search for a paragon should not let him hide from the urgency of his need.

THE new president of the United States may be saying all the right things (see *Nature* 337, 585; 1989), but he is doing fewer of them than he should. This complaint has been levelled generally at the new administration, which has yet to fill its roster of cabinet appointments, let alone all the second-rank posts, all of whose incumbents will have to be interviewed by a committee of the Senate and judged capable of doing their jobs. But the complaint is particularly apt when applied to President Bush's plans for the more effective administration of science, among the most ambitious of those put forward last month.

Briefly, Bush would give a new science adviser the status of "assistant to the President", putting him or her on a par with the head of the National Security Agency (now General Brentt Scowcroft). On top of that, there is to be a Council of Science and Technology Advisors resembling in all but name what used to be the President's Science Advisory Committee (PSAC), much respected until killed off by President Richard M. Nixon. But nobody has yet been nominated for the top job. Even if a name should have appeared before the end of this week, a quarter of a year (or a sixteenth of a presidential term) will have gone before the man or woman can set to work.

The administration's general excuse for its tardiness — that its succession of the like-minded Reagan administration means fewer policy discontinuities and thus a lesser need of new officials — does not apply to the science advisory job which, as advertised, is an innovation. The present holder of the office, Mr William Graham, has had only meagre influence during his two years, and is unlikely to be struggling for promotion.

So why not pick somebody and get on with it? The word is that the administration is looking for a science adviser from industry, which is consistent with the Bush view of science as a means of improving the competitiveness of the United States in the "global marketplace". One snag is the formidable list of loyalty tests the new incumbent will have to survive: finding a Republican will be easy, finding somebody willing to go to the stake for the Strategic Defense Initiative will be something else.

A more practical difficulty may be that there is already a technical person powerfully placed at the White House. Mr John Sununu, Bush's chief of staff, was a chemical engineer before becoming governor of New Hampshire. Between gaps in what must be the fullest appointments diary in the Western world, not to mention side-trips to

Japan, China and Korea, Sununu is interviewing potential candidates for the new job. Quite apart from his lack of time, there may be a further difficulty: some putative incumbents may be deterred by knowing that the man who allocates space in Bush's working day is likely to have his own strong views on what science advice the president should be given.

Yet the need for an appointment becomes more urgent every day. In just over two months, the National Security Council is to have completed a review of US policy on national security, to which an able science adviser would have a powerful contribution to make.

Soon afterwards, early in the summer, there will be a ministerial meeting of the North Atlantic Treaty Organization that will be crucial for the future of US weapons policy. Meanwhile, the administration is being challenged by the daunting character of its own agenda (improving public education, doing something effective about AIDS and drugs) as well as by the conundrums thrown up by the Congress, the still-unfocused worry about climatic change for example (see page 3). The danger is that too many of these issues will be decided by default.

The reconstitution of PSAC will raise other problems. From its creation after the Second World War until its abolition, the old PSAC won respect by the self-evident independence of its members. Sometimes it could be an irritating talking-shop, but its strength was its readiness to oppose administration policy when it thought fit. Bush is looking for a council of "leading scientists, engineers and distinguished executives from the private sector" (which includes the universities), which is not in itself a bad prescription. But will he allow that the advisers should be chosen for the calibre of the advice they have to offer, not the likelihood that the advice will be acceptable? That will be the acid test of whether Bush means all the right things he is saying. □

## Science squeezed

Nobody should be surprised that ten years of short commons have deprived Britain of researchers.

THE emergence in Britain of a shortage of young researchers (see page 7) is about as remarkable as the diurnal rhythm. For close on ten years, British research



establishments have been harassed by lack of funds, so that newspapers have been full of tales of researchers who have been put out to grass or who have fled abroad. Young people, who read the newspapers, have also learned from them that entrants to other professions, that of providing financial services, for example, are likely to live more prosperously, and have voted with their feet. That there is a shortage of putative graduate students is not in the least surprising, but a predictable consequence of the market forces on which the British government bases most of its policies, sometimes successfully. It is even more worrying that the past few years have probably frightened away an unduly large proportion of the most able people.

There is now no quick remedy. The Medical Research Council's plan to offer graduate students on its books an extra £500 a year, commendable enough, is about as relevant to the underlying market forces as would be a great man's attempt to interfere with the diurnal tides by walking into the sea (which is what King Canute demonstrated nine centuries ago). Even doubling the present meagre stipend would have little beneficial effect. Only when the practice of research in Britain has been shown again to be an honoured as well as an honourable profession will young people be clamouring for the studentships on offer.

That calculation should be central in the planning of how to spend the extra sums for research now being made available. But that appears not to have been the case. Too much of the extra is being spent on directed research or, worse, on the research councils' own establishments, too little on the means (research grants) by which individuals might make a mark and thus fire the imagination of young people choosing a career. Researchers should fight to see this balance redressed, knowing that in doing so they will be serving the wider British national interest.

Meanwhile, as a stop-gap remedy, the research councils might think of offering their studentships to young people from abroad, coupled with work permits to stay in Britain permanently. In the United States, for different reasons afflicted by a shortage of young researchers, the stragem works well enough. □

## Satanic violences

The Shiite threat to kill a British author and the violence of animal rights extremists have a lot in common.

MR Salman Rushdie's book *Satanic Verses* opens with the fantasy of how two unremarkable people survive the destruction (by a bomb) of an aircraft over the English Channel and then found a new religion. The ways of the Shiite Muslims who have now offered a money prize for Rushdie's assassination and those of the people who advocate civil rights for animals may seem only remotely connected, but they are nevertheless worth remarking.

Apart from the bomb last week at the University of

Bristol (remarkable only for being the first use of high explosives to damage British property in this particular cause) and the occupation of a crane on a Berkeley construction site, this has been a relatively quiet week, yet in their character the protests on behalf of animals have precisely the qualities that make the threats against Rushdie as offensive as they are.

First, it must be clear that the Shiite threat is an act of violence. One apologist last week is quoted as saying that "the arrow . . . is already travelling to his heart", by which is presumably meant that the threat will be regarded for decades to come as an invitation to some impressionable assassin to push the arrow home. The more tangible violence of the extremists in the animal rights movements has the same implacable quality; nothing, especially not reason, can divert it. It has the same quality of detachment from the identities of those who are injured; Shiites imprison random Western travellers to Beirut for years on end, while the explosion of a bomb at the University of Bristol is similarly random (but there is a veterinary school there). Why pick on that rather than on some other British university? Because it happened to be handy, perhaps?

As with the case of Rushdie so on the animal rights front, the most urgent need is that the moderates who believe that Rushdie's book is blasphemous (to Muslims) or that animals are misused in research should clearly dissociate themselves from the actions of the extremists. At least in the use of animals in research, the moderates have a powerful case, not least in their demands that people's use of animals should be as sparing as possible and that humane procedures should be followed. Failure to condemn these acts of violence has two consequences; the extremists are excited, while the moderate case is so tarnished by its association with violence that it is dangerously weakened. Sadly, that lesson seems not yet to have been learned, either in the case of Rushdie's book or that of the bomb at Bristol. □

## Unfamiliar disguises

The unfamiliar appearance of this issue of *Nature* implies no systematic change of content.

REGULAR readers who happen to notice the typographical changes in this week's format will passionately resent the small changes which have been made. That at least is what past experience shows. The only comfort is that, with the passage of a few weeks, the same readers will become just as vigilant in the defence of what is now offered — a layout which is generally more uniform, in particular respects more workable and which may help to make some sections of *Nature* more readable. No attempt has been made, on this occasion, to introduce fancy typographical tricks or even to restore the couplet from Wordsworth's "Endymion" from which this journal's name was taken in 1869. □

# US Congress plans greenhouse legislation

- Tough measures on global warming
- Scientific opinions differ

## Washington

THE new US Congress has begun with a stampede by legislators introducing bills meant to respond to the perceived threat of global warming. But researchers at congressional hearings last week seemed much more cautious than the legislators in estimating what the future holds for the world's climate.

Several wide-ranging bills on global warming have been introduced since the Congress opened on 25 January. The most recent, the "Global Warming Prevention Act of 1989", was announced by Claudine Schneider (Republican, Rhode Island) of the House of Representatives on 21 February, and has 50 co-sponsors.

Schneider's bill embodies many of the elements of those launched a few weeks ago by Senator Timothy Wirth (Democrat, Colorado) and Senator Albert Gore (Democrat, Tennessee). It calls for an international agreement on a 20 per cent reduction in global carbon dioxide concentrations by the year 2000 and the revision of the Montreal Protocol to phase out all chlorofluorocarbon production within five to seven years. Under the legislation, US aid for family planning services in developing nations is doubled to help reduce population growth (and hence energy demand) and recipients of foreign aid will be required to practise sustainable forest management. Tropical timber imports will be banned from countries that do not comply.

On the domestic front, the bill would provide hundreds of millions of extra dollars for research and development on energy-efficient technology, solar and renewable energy resources and solar-generated hydrogen fuels. There would be tax rebates for fuel-efficient cars, and increased 'gas guzzler' taxes.

The drive to legislate stems from widespread public concern about global warming, and the greenhouse effect. The issue caught the attention of the media during last summer's drought in the United States. But, ironically, few if any researchers attribute the drought to the greenhouse effect. Stephen Schneider of the National Center for Atmospheric Research (NCAR), giving evidence to the House of Representatives' subcommittee on Energy and Power last week, said it is "absurd" to suggest a direct causal link. But he welcomes the attention global warming is receiving in Congress even if it is "for the wrong reasons".

NCAR's Schneider strongly suspects

that the high average global temperatures of the 1980s, which are about half a degree Celsius higher than at the end of the last century, are a greenhouse signal; he advocates action now instead of later.

But Robert Correll, of the National Science Foundation, in evidence last week to the Senate Commerce, Science and Transportation Committee, noted that the increase of temperature over the past century has not been steady, in contrast to model predictions of the greenhouse effect. And Correll says that the current differences of opinion among scientists as to whether there is now a greenhouse

warming are "substantial".

Some members of both committees seemed irritated by the lack of consensus and warned the scientific witnesses that their uncertainty might be used by some congressmen as an excuse for no action.

Regardless of uncertainty, opposition to the new legislation is bound to be strong. The powerful automobile industry will oppose legislation that favours more fuel-efficient imports, and the coal industry will object to action that might substantially reduce coal consumption. The new bills are expected to have a particularly rough passage through the Senate Energy Committee, where these interest groups have a strong voice. But there is considerable public pressure on Congress to "do something", and if the heat waves strike again this summer — particularly if they strike Washington — it is almost certain that some of the new legislation will be passed.

David Swinbanks

■ See also pages 15 and 54.

## US REACTORS

# Shoreham plant to be revived?

## Boston

NEW YORK's never-licensed Shoreham Nuclear Power Station may be rising from its grave, if its parent utility's stock is any indication. The stock of the Long Island Lighting Company (LILCO) has recently been the third most actively traded of the stocks, on the New York Stock Exchange.

The speculative flurry has been caused by two developments in the tangled but continuing saga of the nuclear plant. A federal judge has thrown out a case brought by the local legislature which accused LILCO of "racketeering" and corruption, and the US Nuclear Regulatory

also have raised electricity rates, even though it would have restricted the utility's right to pass on costs to ratepayers (see *Nature* 333, 588; 1988).

The plan was sanctioned by all the major parties involved, but was derailed unexpectedly when the New York State legislature refused to ratify it out of fear of political reprisals.

Ironically, Long Island constituents, whose electricity rates are already the second highest in the United States, will now face even greater cost increases, whether or not the plant goes into operation.

There are still many hurdles ahead for the Shoreham reactor, most notably the strong local and state-based political opposition.

Shoreham's local Suffolk County legislature voted unanimously to appeal against the rejection of its racketeering and corruption suit against LILCO, turning down a settlement of nearly \$400 million offered by the utility in reduced electric rates. The legislature said it would not settle the case unless the utility promised that it would "irrevocably close, shutdown and decommission" Shoreham.

But despite their differences, all the parties involved agree that Shoreham's fate is highly uncertain. Even LILCO representatives say that they are now following a "two-track" strategy: moving ahead to license the reactor, and trying to negotiate a scheme to shut the plant down. What they find intolerable is the waiting and indecision, which they claim has cost the utility \$1,700 million in interest and finance charges since the plant was completed.

Seth Shulman



Commission is expected to rule soon upon whether to grant the Shoreham reactor a low-power operating licence. Both developments promise a future for the power station, which was completed four years ago.

The Shoreham plant was widely pronounced dead last spring, when New York State authorized a plan to close and decommission the reactor, but which would



## UK ANIMAL RIGHTS

# University building bombed

## London

BRITISH police now fear a bombing campaign against other universities after the explosion of a bomb, thought to have contained 5 pounds of high explosives, in an administrative building of the University of Bristol last week. The bomb went off in the staff restaurant of the Senate House at midnight on Wednesday, 22 February. There were no casualties.

Responsibility for the attack has been claimed by the Animal Liberation Front (ALF), a well-known group of extremists, and by two previously unknown groups, the Animal Defence Organisation and the Animal Abused Society, which warned the police beforehand of the bomb. The ALF has usually concentrated its attacks on the houses of scientists, which have been daubed with paint, and on retail shops selling items such as furs.

The Secretary of State for Education and Science, Mr Kenneth Baker, visiting the university after the explosion, called the attack "an appalling example of terrorism". He said that tighter security might be necessary at British universities.

British animal rights groups have previously had some success with incendiary



Bomb damage inside the Senate House.

devices, but have not hitherto used high explosives. Animal rights groups which campaign using non-violent means against vivisection have condemned the bombing. A spokesman for the British Union for the Abolition of Vivisection says it will cause extreme damage to the campaign against animal experimentation. There are 50,000 members of non-violent groups, he says, and less than 100 in the loosely-organized group of individuals which makes up the ALF.

Christine McGourty

## US ANIMAL RIGHTS

# Activists setting the agenda

## Berkeley

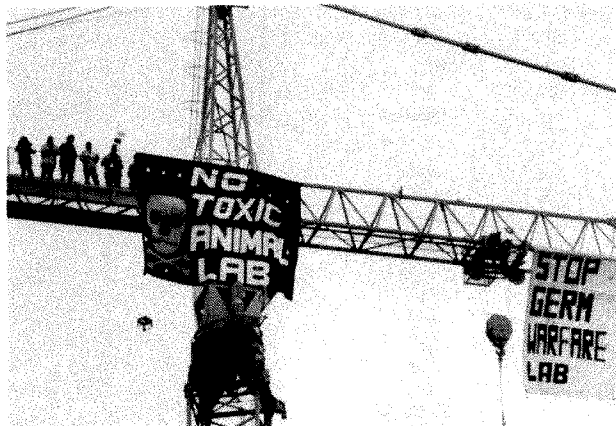
THE divisive issue of the use of animals in research was the centre of attention last week at two San Francisco area universities. Stanford University has been debating a genteel change of policy that would affect the use of animals in teaching, while, on the University of California campus at Berkeley, a more acerbic debate over an animal care facility has flared up.

Animal rights activists have been stepping up their activities in California. Last week, six of them scaled a 175-foot crane at a construction site on the Berkeley campus to protest at the building of the

university's new animal facility. Unfurling banners that read "Stop Germ Warfare Lab!" and "No Toxic Animal Lab!", the protesters vowed to remain on the crane for two weeks. But university officials have called the stunt "ironic", because the crane is not involved in the construction of the new animal facility, but instead is being used to put the finishing touches to a plant-biology building.

The university is hesitant to remove the protesters, fearing that somebody would be hurt in the process. Construction of the animal facility has continued undisturbed, but the protest is costing the university more than \$10,000 a day, according to one spokesman, to pay for 24-hour security guards and lost construction time.

Meanwhile, in an effort to avert future animal rights controversies in the classroom, Stanford University has drafted a policy that would inform students which courses and degree programmes use animals in instruction. The new policy, now being finally revised, encourages students to raise with their instruc-



High-level protests against Berkeley's new laboratory.

David Maung/Daily Californian

## SOUTHERN AFRICA

# UCT again confronts government

## Oxford

THE University of Cape Town (UCT) is once again in confrontation with the South African government, this time because of a breach of confidence by the Minister of Health and Population Development, Dr Willie van Nierkerk.

The issue concerns Dr Jocelyn Kane-Berman, whom van Nierkerk sacked from her former post as superintendent of the Groote Schuur Hospital, UCT's teaching hospital, and the post of dean of the medical school at UCT, which the university is now seeking to fill against a pending retirement. In reply to parliamentary questions about the dismissal, van Nierkerk said that he would approve Kane-Berman's appointment as dean because, at UCT, she would be "in an environment where the proximity of the ANC (African National Congress) is well-known".

Apart from the slur on the university, UCT is incensed that van Nierkerk should comment publicly on one application for an academic post which is still under consideration. Van Nierkerk, a former professor of obstetrics and gynaecology at Stellenbosch, can hardly be ignorant of the niceties of academic appointments.

UCT now says that the insinuations in the minister's remarks are "disgraceful". Dr Stuart Saunders, the vice-chancellor, and Mr Leo Abrahamse, the chairman of the council, who met the minister to protest, said that van Nierkerk had chosen not to provide an explanation, and that the university would now be making representations through other channels.

The cause of Kane-Berman's sacking from Groote Schuur was her statement, to a weekend newspaper, that Nelson Mandela would be her choice as prime minister in a South African government chosen on merit (see *Nature* 336, 612; 1988). The *South African Medical Journal* has called for her reinstatement, but the minister is unrepentant. Meanwhile, Kane-Berman intends to sue her former employers, the provincial administration, for reinstatement.

Michael Cherry

tors any concerns they may have about animal use. But although it provides room for individual solutions, the policy does not oblige instructors to change course requirements.

Stanford spokesman Robert Beyers said the concept of forewarning students was stimulated by the passage in the state legislature last spring of a law that allows primary and secondary school students to refuse to dissect animals in the classroom.

The new rule may have little impact, because animals are rarely used in undergraduate courses, and no required courses in the medical school or the biology department involve animals.

Marcla Barlnaga

## SUPERCONDUCTOR PATENTS

# Four groups join battle

## Washington

THE US Patent Office is now wrestling with competing claims to patents on high-temperature superconductivity from four different research groups — at IBM, AT&T, the University of Houston and the US Navy. The patent office says there is a conflict over priority in the four claims to have discovered useable versions of the copper oxide superconductors. If the patents office can resolve the conflict, its decision could be a boon for the organization whose claim succeeds.

The conflict, legally called an 'interference' proceeding, has arisen because the patent applications by the four are similar, overlapping, and were filed at almost the same time. The parties to the dispute have each filed a string of patent applications for both the lanthanum copper oxide materials discovered in 1986 by Nobel prizewinners Georg Bednorz and Alex Müller, of IBM Zurich, and the yttrium-based copper oxides discovered by Paul Chu of the University of Houston and Maw-Kuen Wu of Alabama University in January 1987. It is widely believed that the yttrium compounds will have more general commercial application than the lanthanum materials.

The US Patent Office will have to decide not only whether patent applications for the lanthanum oxide superconductors can be extended to cover the yttrium compounds, but also whether applications based on mixed-phase materials of unknown crystal structure are valid (as in the case of Chu's first patent application for yttrium copper oxide material).

Information about patent applications for the superconductor materials began to come to light last summer, in Europe and Japan, where by law applications are made public 18 months after submission (or after the earliest priority date claimed). US patents are not published until granted, but several of the European and Japanese patent applications include priority claims for US patents and so disclose US filing dates.

The application from the US Naval Research Laboratory has not previously been known of, having been filed only in the United States. At the applications stage, even cases of interference are not publicized by the US patent office, but Cynthia Stevens, an IBM spokeswoman on patents, confirms the identity of the four competing applicants.

Japanese patent applications are notable by their absence in the dispute, in contrast with the common fear in the United States that Japan will be the first to exploit the commercial applications of the high-temperature superconductors. One explanation may be that the early patent applications by University of Tokyo

researchers are narrowly drawn and cover only the lanthanum oxides (see *Nature* **335**, 389; 29 September 1988).

Japan's Ministry of International Trade and Industry (MITI), which has filed broad applications worldwide covering the yttrium oxides, may claim interference at a later stage, according to Christopher Vear of the British Technology Group who is monitoring patent applications for the new materials. But MITI filed after the US groups in January 1987 and does not seem to have a strong position.

Japan's real strength lies in patent applications for devices made from the new materials and processes for making the superconductors into wires and thin films. Japanese companies, in particular Sumitomo Electric Corporation, have filed hundreds of such applications in Japan and many have been combined into broad patent applications in Europe, where Japanese interests have filed most applications. According to Vear's latest figures, Japan has filed 80 applications in Europe compared with 28 for the United States, 5 for West Germany, 4 for Britain, 3 for France and 1 each for the Soviet Union and Hungary.

The only patent so far granted has been awarded by the US Patent Office to John B. Vander Sande and Gregory J. Yurek at the Massachusetts Institute of Technology (MIT) for a process that combines the new ceramic materials with silver to make a more malleable material (see *Nature* **336**, 607; 15 December 1988). But soon after the award was announced, Sumitomo filed a claim for interference with a similar patent application it had filed within a day of the MIT researchers, according to Vear. Such claims of interference can be expected to multiply dramatically in the near future.

Patent interference cases in the United States can take years to resolve. One example is the battle over patent applications for the gas discharge laser, which took 28 years. The dispute over patent rights on high-temperature superconductor materials may become such a case because of the large number of parties involved. But a long legal battle could work to the eventual winner's advantage. If, as many experts predict, commercial applications of the new materials will not be feasible for at least a decade and because, in the United States, the life of a patent begins only after it is issued, the group to which the patent is eventually granted may enjoy licence fees for longer than if its claim had never been challenged.

**David Swinbanks**

■ On page 49 of this issue, the recently developed 'electron' superconductors show behaviour similar to the more established 'hole' superconductors. □

## NEWS IN BRIEF

## Personal communication

### London

THE Union of Scientists and Inventors' Societies of the USSR has established a special "international commission for relations with former compatriots", aimed at establishing links with Soviet scientists and engineers now working in the West. Such people have hitherto been regarded as ungrateful renegades, who have accepted the benefits of a Soviet education and then decamped to the capitalists. The new commission, however, considers them a potential channel for establishing closer cooperation between Soviet science and the West. Interviewed by *Sotsialisticheskaya Industriya*, commission member Academician Yuri Gulyaev suggested a whole range of ways in which the *émigrés* could help Soviet science, from taking Soviet graduate students into their laboratories for a year or two to organizing joint research groups. Many countries, Gulyaev said, gained considerably by working with their *émigré* scientists in this way — Hungary especially. "We have been accustomed to working only through official channels" . . . but personal contacts and informal links are sometimes more effective." V.R.

## Three per cent is six

### London

BRITISH universities will receive a 3 per cent increase in government grants for recurrent expenditure in 1989–90. The minister for higher education, Robert Jackson, announced that £1,680 million will be distributed to the universities in the next academic year, an increase of 6 per cent from 1988–89, but much of the increase included in the government figures is accounted for by costs for restructuring and agreed pay awards for previous years. The universities' vice-chancellors say the 3 per cent increase is too small, and have asked for an increase of 5 per cent. Jackson also said the universities should plan for an increase in 1990–91 of 4 per cent in cash terms and increases of 3 per cent in the following two years. C. McG.

## Chip complex destroyed

### New Delhi

INDIA'S microelectronics industry has been shaken by a fire that almost totally destroyed the \$100-million semiconductor complex in Chandigarh, the country's only facility for producing silicon chips for civilian needs. Sabotage has not been ruled out as the cause of the fire, which burnt down the entire complex, leaving only some design documents and the administration block. Mr Virendra Mohan, chief of the facility, has resigned after acknowledging moral responsibility for the accident. There are now fears that many of the nearly 200 engineers working at the Chandigarh complex and who are now out of work may migrate to the West.

India's semiconductor needs will now have to be met by imports or by setting up a new facility that will not be ready before 1992.

K. S. J.



## Oceanographers' lobby

Washington

To help convince Congress that oceanographers should play an important role in plans to address the issue of global change (see page 3), representatives from some four dozen oceanographic institutions met last week in Washington to form the Council on Ocean Affairs.

Jim Baker, president of the Joint Oceanographic Institutions, which has sponsored the new council, says that while federal agencies such as the NOAA and NASA are knowledgeable about the needs of oceanographers, Congress needs independently to be educated about the issues important to them.

While the council is intended to represent institutions, the new Oceanography Society, also promoted by Baker, is a professional society for oceanographers. The society will hold its first annual meeting this summer in Monterey, California. J.P.

## Staab steps down

Munich

HEINZ Staab, president of West Germany's Max Planck Society (MPS), has announced that he will resign when his six-year term ends in the summer of 1990. A newly formed selection committee will present a list of potential replacements to the MPS senate in November.

Staab, 62, who is also a director of the Max Planck Institute for Medical Research at Heidelberg, would have been eligible for one more term. He is stepping down to devote himself to research. S.D.

## Melbourne rivalled

Sydney

SYDNEY may be about to take a step to right the balance between itself and Melbourne, traditionally the centre of Australian medical research.

Professor Tony Basten has been appointed director of the new Centenary Institute of Cancer Studies and Cell Biology, a joint venture between the Royal Prince Alfred Hospital and the University of Sydney. The new institute will incorporate the Clinical Immunology Research Centre at the University of Sydney and is due to move a staff of 150 to two floors of a new six-storey building by October 1990.

The institute will be supported by state and federal grants, a block grant from the National Health and Medical Research Council and money from the private sector. T.E.

## Genentech's heart drug

Washington

THE US Food and Drug Administration ruled on Monday of this week that Genentech can claim in its advertising that TPA (tissue plasminogen activator) reduces the death rate among patients experiencing a heart attack, based on new data from two ongoing clinical trials comparing several heart drugs. Previously, the agency would only allow that TPA treatment dissolves the blood clots causing the heart attack. C.E.

# Freeze hits BMFT research

Munich

BATTLE lines have been drawn for the fight for next year's budget for the West German Research and Technology Ministry (BMFT), with European space projects, microelectronics and other politically sensitive items hanging in the balance. The federal research minister, Heinz Reisenhuber (Christian Democrat or CDU), has even come under attack from his own party as well as the opposition for not fighting harder for an increase in his stagnating budget.

BMFT is responsible for the general promotion, planning and coordination of research in West Germany. It supports technological research and development, data processing, nuclear research and technology as well as space and aeronautical research. As well as providing the lion's share of support for the Large Research Establishments (*Grossforschungseinrichtungen*) and the Max Planck Society, BMFT supports basic research at universities in selected areas.

The BMFT budget will decrease in real terms in 1989 for the first time in recent memory, if a budget freeze imposed last November is not lifted. The ministry lost DM190 million of its DM7,600 million budget to a "global reduction" imposed on all ministries. A further DM166 million has been frozen by the finance minister. Coupled with the effects of inflation, these cuts result in a reduction of the overall amount spent by BMFT in 1989, instead of the 2.8 per cent increase announced last year.

Ministry officials are not optimistic that the freeze will be lifted, but are trying to estimate where the axe will fall. A likely candidate for a cut would be the West German contribution to the large projects of the European Space Agency (ESA). But ESA would have to approve any such reductions, the effect of which might be to delay projects such as the space shuttle Hermes or the booster rocket Ariane 5.

The Max Planck Society, 60 per cent of whose funds derive from BMFT, will be largely protected from the cuts, thanks to the efforts of the West German finance minister Gerhard Stoltenberg and the *Länder* (states).

The shape of the 1990 budget will emerge in the next few weeks from discussions between BMFT and the Finance Ministry. The government will present a budget to parliament only in July, after its broad scope has been decided behind closed doors.

Meanwhile, there are signs that the ministry will shake off its lethargy and, even at this stage, ask for a big increase in 1990, partly to make up for the cuts in

1989. A ministry spokesman would not say how big an increase Reisenhuber will seek, except to say that it will be more than the growth of the overall federal budget. Some predict that the minister will ask for an extra 6 per cent.

West German participation in Hermes and the space station Columbus has begun to look even less certain than before in the light of the struggle over the BMFT budget. The consensus in Bonn is that it will probably not matter if a few tens of millions of deutschmarks are delayed until next year. But a much bigger commitment will be necessary in 1991 if Hermes and Columbus are to get off the ground. So far, the money required, eventually amounting to thousands of millions of deutschmarks, has not shown up in the medium-term West German budget.

In a related development, the Bundestag budget committee voted last week to release a previously frozen DM27 million for supersonic transport technology. The money will be invested in developing the proposed West German space plane Sänger, which represents the next generation of space shuttle technology.

Yet West German research and development continues to grow. But figures made public by BMFT show that the rising investment in West German research and development derives primarily from industry. No less than 74 per cent of the

Heinz Reisenhuber (right) is under pressure from his own party and the opposition. Social Democrat member Josef Vosen complains "Reisenhuber has promised a lot, but the money is not [yet] there".



DM61,400 million spent in 1988 was industry money. But a BMFT official says it is "questionable" whether overall research spending will continue to grow as fast as the gross domestic product, given the slow growth in the BMFT budget and the loss in 1990 of tax deductions for some company research.

BMFT notes that the trend elsewhere, in Japan and the United States for example, is just the reverse, with governments spending "disproportionately much" on research. But a BMFT official adds that there has recently been a "storm" of grant applications from university researchers, suggesting that the stiff competition for grants experienced by the Deutsche Forschungsgemeinschaft (DFG) has now hit BMFT as well (see *Nature* 337, 590; 16 February 1989).

Steven Dickman

## SWISS RESEARCH

# OECD urges changes for 1992

## Munich

SWEEPING changes in science and technology policy will be required if Switzerland is to meet the challenge of the internal European market planned for 1992. That is one conclusion of a report\* released by the Paris-based Organisation for Economic Co-operation and Development (OECD) in Berne this week. The review was carried out at the request of the Swiss government.

The survey team of three international examiners based their report on a series of site visits to Swiss laboratories, universities and public and private institutions.

The recommendations urge changes in policy at federal and cantonal levels as well as in universities and in industry. The reviewers found a "distressing" lack of cohesion in national science policy, with a resulting parochialism and slowness to change. Such traits may leave Switzerland "almost isolated in Western Europe" given the country's decision to remain outside the European Community (EC).

The most striking recommendation is the creation of a "secretary of state" for science and technology in the Federal Department for Home Affairs. This official would coordinate federal and cantonal policies as well as Swiss participation in international programmes.

The OECD report encourages Switzerland to invest more in research and to do so more effectively. In spite of being the richest of all OECD member countries, the report says that "public resources for research do not match those of most other OECD countries". The high percentage of the gross domestic product invested in research comes about because of research-intensive pharmaceutical and electro-mechanical industries. OECD strongly recommends that the Swiss government streamline the diffuse structure for research policy-making and establish a "national research budget" — at present decisions are made at a local level in the departments.

The lack of major public research institutions (with the single exception of the Paul Scherrer Institute for nuclear research) comparable to those elsewhere in Europe gives the team the impression of "weakness, dispersal and fragmentation of public R&D funding," said OECD.

Among the new programmes recommended by OECD are "substantial" national research programmes in information technology, new materials and biotechnology. The lesson learned when Switzerland "missed the turning" on the electronics road — and failed to develop an indigenous industry in digital watches or other electronic products — must not

be forgotten, warned OECD.

OECD concurred with some university researchers and industrialists (see *Nature* 336, 331; 24 November 1988) that Switzerland needs a publicity campaign explaining the benefits of science to the public and to school leavers. Distressingly few Swiss students are studying science and engineering, raising doubts about the future supply of skilled personnel.

On the Swiss National Science Foundation, which supports much of the basic research at Swiss universities, the report called "unhealthy" the practice of reducing individual project allocations by up to 30 per cent to allow more proposals to succeed. This encourages groups to submit inflated grant proposals and can prevent some projects from achieving "critical mass."

## RESEARCH COUNCILS

### MRC offers more

#### London

THE British Medical Research Council (MRC) wants to increase the pay of graduate students in the hope of stemming the growing shortage of young researchers. Last week, the council asked the Department of Education and Science (DES) to allow an increase from just under £3,000 to £3,500 in the stipends paid to the holders of its basic research studentships from next September. In doing so, the council is planning to break with the traditional method of calculating graduate students' pay using a DES formula linking it with the maintenance grants for which undergraduates are eligible.

The council also wishes to offer an extra £500 a year to graduate students working on its directed AIDS programme of research, which would be the first time that pay differentials not based on location had been offered. The stipends paid to holders of research council studentships are usually tax-free.

The problem of attracting talented young researchers is also worrying the Advisory Board for the Research Councils. At a meeting organized by the Royal Society last week, ABRC chairman Sir David Phillips said that the shortage of research manpower would be the most serious problems the research councils would have to face in the 1990s.

ABRC's Biotechnology Advisory Group is now urging the board to set up a formal review of the manpower shortage and to suggest ways in which it might be remedied. One member of the group, Professor Peter Dunhill of University College, London, says that if nothing is done about the shortage, all the councils' initiatives in biotechnology could fall apart.

Christine McGourty

The report repeats the commonly voiced fear that, without subsidies, the small and medium-sized companies forming a large part of Swiss industry will not be able to compete with their rivals in EC countries.

Rounding out the OECD recommendations is a list of conditions to be met if Switzerland's "production apparatus" is to face the needs of modernization. The list was first prepared by the Battelle Institute in 1983 in a study of what had gone wrong with Swiss industry in the 1970s. The Battelle Institute said Swiss industry should take more risks in introducing new technology, that large and small companies should cooperate more closely, and that the banks should take a greater interest in the promotion of new technology. OECD said that the recommendations are "still valid today."

Steven Dickman

## NAGYMAROS DAM

### Objectors find a voice

#### London

THREE members of the Hungarian National Assembly are in danger of losing their jobs because of their electors' objections to the controversial Gaboikov-Nagymaros hydroelectric scheme. After a majority of the assembly voted for completion of the Hungarian part of the scheme, three constituencies (two in Budapest and one in Szeged) have voted to recall the mandates of their representatives under a previously unused provision of the Hungarian constitution.

The decisions reflect growing opposition in Hungary to the Nagymaros dam, part of a joint project with Czechoslovakia. There are demands that the government should annul its treaty obligations to Czechoslovakia (as well as the construction debt to Austria), and that Hungary should pull out of the scheme unilaterally.

The decisions also reflect a new political awareness in Hungary. In a one-party system (as at present), the right of recall is a guarantee that constituents' views will be accurately represented in the assembly. (A fourth deputy is threatened with recall for reasons unconnected with the dam.) But there are signs that the ruling Socialist Workers' Party may abdicate its monopoly; the Central Committee has now accepted the principle of a multi-party system with appropriate "checks and balances".

But whether this will allow the emergence of a 'green' party in Hungary is uncertain. Janos Vargha, one of the leaders of the 'Danube circle' which has campaigned against the dam told a recent conference of environmentalists that a green party could compromise the independence of existing groups.

Vera Rich

\*Reviews of National Science and Technology Policies: Switzerland. The report, including an account of the review meeting held in Berne on 3 March, will appear in book form later this year.



## REMOTE SENSING

## European satellite reception

### Paris

THERE is now hope that the European Remote Sensing Satellite, ERS-1, to be launched next year, will have an enthusiastic band of users. That seems to be the outcome of last month's meeting between the director-general of the European Space Agency (ESA), Reimar Lüst, and the President of the Commission of the European Communities (EC), Jacques Delors.

The satellite will allow Europe to have a major role in studying the world climate and the effects of pollution. But although the infrastructure of ground stations to receive data from ERS-1 is already in place, the network of potential users is still relatively underdeveloped but last month's meeting seems to have helped.

ERS-1 will also be at the heart of the World Ocean Circulation Experiment (WOCE), which will come alive, after 10 years of preparation, with the satellite's launch. Its advanced radar instrumentation will allow accurate data to be collected on sea surface temperatures, ice flows and ocean circulation, as part of an international effort to understand global

climate and natural disasters. The satellite could also be used to monitor the rate of destruction of tropical rain forests, often invisible to other remote-sensing satellites because of cloud cover.

The meeting between Delors and Lüst was aimed at soldering links between ESA and the European Community. "The European Commission is like a dream client", said Jean Arets, head of international relations at ESA. One possibility seems to be a high-level working group to encourage environmental monitoring by satellite. Arets also says that the European Community could help to finance the creation of a community of users.

The goals of ESA and the EC are reciprocal. ESA is keen to improve its coordination with the EC's new technology programmes, while the EC wishes to strengthen pan-European involvement in ESA programmes for Earth observation. It also seeks a long-term strategy to exploit microgravity techniques, and has an even more immediate need of common norms for telecommunications.

Peter Coles

## AURORAL SATELLITE

## Japan continues in an old furrow

### Tokyo

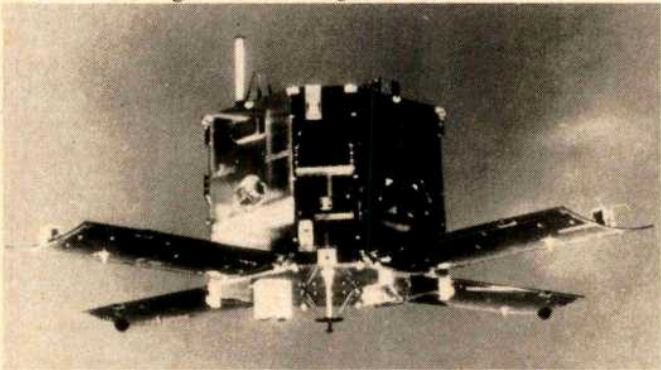
THE first satellite designed specifically for study of the particle acceleration mechanisms producing the aurora borealis (and australis) was launched successfully last week (22 February) by Japan's Institute of Space and Astronautical Science (ISAS) from the Kagoshima Space Center.

The EXOS-D satellite was carried into a polar orbit by a Mu-3S II three-stage solid-fuel rocket, independently developed under ISAS's scientific space research programme. The 300-kg satellite, given the post-launch name of 'Akebono' (Dawn), will fly into the region where charged particles from the solar wind are accelerated by the Earth's magnetic field and penetrate to the upper atmosphere, causing auroral displays. The orbit will take the satellite from a near point of 300 km from Earth out to 10,000 km.

On board Akebono are instruments to monitor magnetic and electric fields, plasma waves, low-energy particles, supra-thermal ions and thermal electrons as well as a special auroral camera

designed to take photographs in the visual and ultraviolet spectrum. Early next year, ISAS hopes to add to Akebono's view by launching sounding rockets capable of reaching a height of 350 kilometres from a base in Norway, making it possible to obtain the first simultaneous observations of the aurora from above and below.

Akebono is ISAS's 12th scientific observation satellite and the third in a series designed to look at the aurora and



Akebono: a first in aurora research.

magnetosphere, following on from Kyokko ('Northern lights') and Jikiken ('Magnetosphere'). ISAS plans to continue providing a key role for Japanese researchers in solar wind and magnetospheric research with the launch of a satellite into the Earth's magnetotail in 1992 as a part of the International Solar Terrestrial Physics programme. Alun Anderson

## TOKYO UNIVERSITY

## Close call in election fight

### Tokyo

A TOUGH election battle for the presidency of the University of Tokyo resulted in victory last week for Professor Akito Arima, one of the chief proponents of major change for Japan's leading university.

The election was so close that the university was eventually compelled to choose between two candidates by asking them to draw straws (actually a pair of bamboo sticks with concealed, differently coloured tips). It is the first time that this chance procedure has had to be used in the 112-year history of the university.

Arima (58), a theoretical nuclear physicist, will become president of the university for a four-year term beginning on 1 April. He is the first president to come from the science faculty for more than thirty years and faces a university which is divided over the plans for radical educational reform led by the science faculty.

The science faculty's plans involve fusing undergraduate and graduate schools into a new six-year school (see *Nature* 330, 597; 17 December 1987). But opponents fear it may lead to a drop in prestige for the College of General Education, whose faculty provides the first two years of education to all students and less-specialized further education in departments of Liberal Arts and Pure and Applied Sciences.

Department of Liberal Arts' Professor Nagoya Homma, who has opposed the reform plan, proved to be Arima's chief opponent.

To begin with, there were five candidates and after three rounds of voting the two top candidates both drew exactly 586 votes from the assembled professors, associate professors and lecturers of the university.

At that point, previously unused university rules specified a decision by lottery and Arima drew the winning red-tipped stick.

"Exhausting" was how one Senate member described the day-long election. But a much longer campaign lies ahead for those favouring reform. The members of the science faculty have seen top university research institutes turn into independent national facilities with excellent equipment and have seen private companies set up basic research institutes with large financial resources. Without some strengthening of the university research system, the science faculty are apprehensive that they will find themselves seen as a source of trained students rather than as leading basic researchers.

Alun Anderson



## COMPUTER VIRUSES

# No immunity for Moscow

## Moscow

COMPUTERS in the Soviet Union have been suffering from virus infections just as in the United States and Western Europe. In one of the most recent cases, at the Institute of Applied Mathematics of the Soviet Academy of Sciences, researchers found that some of their programs were disappearing for no apparent reason shortly after retrieval. Their computers had been attacked by a virus. The rogue program was quickly found and killed.

Virus infection is not new. About 20 years ago, programmers at a research institute were annoyed by the erratic behaviour of an ICL computer. The institute was on the point of sending for help from the British supplier when a young programmer admitted having written a program that fooled the operating system while remaining invisible.

With the advent of personal computers, a little too late in the Soviet Union, programmers have been confronted with a formidable job of developing anti-viral codes. The first Soviet machines to suffer from viral infections, ostensibly imported from the West, were Atari computers, followed by IBM-compatible machines.

By the time the International Computer Club was formed in Moscow last December, Soviet programmers had compiled a list of lethal files, including viruses and so-called "Trojan" programs. A user with such a list can identify the infection but cannot cure it. It takes a special anti-viral program to purge the virus.

There are now some 40 virus programs on the Soviet danger list and the number continues to grow. The computer epidemic is spreading because of the woefully inadequate software distribution system in the Soviet Union — or rather because of the lack of any such system. The result is that most Soviet programmers are engaged in entirely disorganized computer activities, seeking casual contacts with colleagues.

With no official software market, such contacts are the only way to obtain software in high demand. Among several hundred of my acquaintances who work with software best-sellers, only a handful have bought them legally.

There is no evidence that specific Soviet computer viruses have been, or are being, developed. Indeed, the best Soviet programmers are said to be working on a computer 'vaccine'. Such a program was described by the central Soviet newspapers last autumn. The idea is that the program identifies a constant part of a known virus and removes it from the host program. But such programs are not universal, as they purge only well-known viruses with 'recognition sites'.

Apart from rudimentary restriction programs, there are two anti-viral 'vaccines' in the Soviet Union. The first is a resident program monitoring a computer's 'abnormal' behaviour, which may suggest that a virus attack is under way. The other may seem more attractive to users in that it is not memory-resident and may be run after a series of operations to give a programmer a list of files which may have been modified by a virus.

Having analysed the suspicious programs the programmers can tell whether the computer system has been infected. One difficulty is that the incubation periods may be very long so that the owner of the damaged machine may discover a new infection.

Worse still, until recently there was no popular magazine for programmers, so that news about viral attacks was spread by word of mouth. The most lethal viruses, which are spread by networks, pose no danger to Soviet computers; there are almost no computer networks in the Soviet Union. Meanwhile, the need for computer virologists and for joint efforts with Western colleagues is increasingly evident.

**Pavel Pevzner**

*Novosti*

## ANTARCTICA

# Greenpeace's claims refuted

## Paris

THE director of the research mission of the French department for Austral and Antarctic Territories (TAAF) has rejected claims by the environmental group Greenpeace that an airstrip being constructed in the French Antarctic Territory contravenes the Antarctic Treaty (see *Nature* 337, 106; 1988). Bernard Morlet says the airstrip is being built to support scientific research, and that steps are being taken to ensure that colonies of Emperor and Adélie penguins are not endangered by construction work.

Plans to build an airstrip at Dumont d'Urville date back to 1983, and are prompted by seasonal restrictions on sea access to the French base, which is surrounded by several hundred square miles of pack-ice in winter. Ships can first reach the base at mid-summer in December, and researchers arriving in January, when the base has been made habitable, have only a month to collect data if they are to avoid being icebound. An airstrip, says Morlet, will allow researchers to winter at the base and to begin work — in human and animal physiology, glaciology and climatology — once summer begins.

According to Greenpeace, the airstrip has destroyed breeding grounds for a colony of Adélie penguins and blocked the migratory path of Emperor penguins. But Morlet says that the migratory path of the Emperor penguins is variable, so that the construction may pose no problem at all, and that, in any case, they are used to climbing onto sea islands to avoid winter storms and so should be able to surmount the airstrip, which is only 5 metres above sea level.

Morlet does acknowledge that the breeding ground of the Adélie penguin, jeopardized by the landing strip, is a problem, but says that the effect of the construction project can be minimized by protecting the adult population and encouraging a change in breeding. He says that the material used for the airstrip may help to create new breeding sites. He also accuses Greenpeace of misreading the Antarctic Treaty. While its third (1983) revision forbids harmful interference with bird and seal colonies — by the use of explosives or by disturbance during their breeding periods — it also allows these activities "...to the minimum extent necessary for the establishment, supply and operation of stations".

Morlet also rejects Greenpeace's allegation that the airstrip is a foot in the door for mineral exploitation. He says that the 2,000-metre icepack beneath the French base makes it unsuitable for this purpose.

**Peter Coles**

# Gorbachev's first Chernobyl visit since disaster

Associated Press



Mr Gorbachev and his wife Raisa pictured talking to workers during a visit to the Chernobyl nuclear plant last week to inspect the site of the world's worst nuclear disaster three years ago. Mr Gorbachev promised that nuclear power, while essential, was unthinkable without guarantees of complete safety — a sensitive issue in the Ukraine, which has the greatest concentration of nuclear power stations in the Soviet Union.



# Human space flight

SIR—The maxim quoted again by G. B. Field, M. J. Rees and D. N. Spergel in their otherwise excellent article (*Nature* 336, 725–726; 1988) that human space-flights “cannot be expected to yield a return commensurate with their cost if the judgement is made in purely scientific terms” is objectionable.

We need look no further than the Viking missions to Mars to see the problems of trying to automate science. Even after the expenditure of \$1,000 million (more than \$3,000 million in current dollars), nobody knows today whether or not there is life on Mars. The Viking landers failed in the single purpose for which they were built. This is not to say that they did not serve other ancillary functions, but had the landers not been constructed around elaborate biology packages, the initial reconnaissance of Mars and its system could have been accomplished for a small fraction of what was actually spent.

We forget that the Viking missions were not inexpensive relative to manned flights. Typical modern estimates for the cost of sending people to Mars (which are little changed from those during the mid-1970s) range from an unrealistic low of \$10,000 million to a more likely \$40,000 million — only 3.3 to 13.3 times the \$3,000 million cost of Viking in current dollars. Can anyone seriously argue that putting a few intelligent and flexible human beings and their equipment on the martian surface for weeks (or for months or years) will result in only thirteen times Viking’s scientific return?

Given the decisions that face the US space programme, a more relevant way to look at this issue is to consider that, even given the highest estimates for the true cost of launching a Spacelab shuttle mission (about \$500 million), the Viking expenditure would pay for no less than six Spacelab flights. Whichever way we measure it, even one Spacelab flight achieves far more science than did Viking. Does it not make sense to consolidate our position in the Earth–Moon system before we go gallivanting across the Solar System? Perhaps this could be done by building the much-maligned space station, which, in addition to laying the technological foundations for human planetary exploration, would admirably serve almost every field of space science but planetary exploration. The space station would extend all the advantages of Spacelab at a cost of only some eight times Viking.

The distinction between reconnaissance and science may seem fine, but I believe it is the natural division between what should be expected of automated spacecraft and what must wait for human

exploration. The lesson of Viking is that, for the foreseeable future, automating creative science will at best be extremely expensive and difficult, and probably impossible — whether on Earth, in orbit or far across the Solar System. This is a lesson we should consider very carefully before spending vast sums of money (\$16,000–10,000 million is the current guess) on a Mars Rover which roves less far with every study, trying to automate what a human driver could do far better for only a few times the cost.

Automated missions are tremendously valuable for some limited applications. But human missions are necessary for most sciences beyond initial reconnaissance, and planetary scientists do themselves no favours by denying that.

DONALD F. ROBERTSON

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## The test of time

SIR—I have wasted a lot of time in my life trying to reproduce astonishing results. I know of scientists who, on any subject, publish in parallel two or three competing interpretations or theories. And a few years ago I was amazed to hear a scientist prominent in my field disclaim five articles he had recently published. It is difficult to convey the resulting scepticism about the literature to young scientists without discouraging them.

I therefore propose that when a scientist is under consideration for a new post or for promotion, his or her publications list should be checked to see whether the findings and interpretations have stood the test of time and, if not, whether they have been retracted. This complements the Harvard rule whereby only positive evidence (the four best papers) is considered. This would uncover quite a few prominent researchers whose latest sensational work always seems to obscure past errors.

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## Rough justice

SIR—William McBride has some valid comments in his letter on the inquiry into his alleged fraudulent conduct (*Nature* 336, 614; 1988). On the basis of his statement that he was not allowed legal representation, nor to attend all the hearings, nor to cross-examine witnesses, he did not receive natural justice.

The transcript of the inquiry is not publicly available and analysts have only

the report to guide them. It seems that McBride should perhaps have been given the benefit of the doubt on having altered the oral dose rates because he believed he was correcting them in answer to a referee’s comment. The report makes a convincing case for finding that McBride did not use “proper scientific method” (*Report* 25; 1988) in his work. This might explain his dubious method of corrections and his incorrect statements about controls. It seems, however, that there was “deliberate falsification” (*Report* 24; 1988) about the number of experimental rabbits.

The final version of McBride’s paper, published by an Australian journal after its rejection by an overseas journal, should have been scrutinized more carefully by the referee in order to remove unsupported conclusions. Foundation 41 and its research advisory committee also performed badly, especially in their failure to investigate fully the staff allegations and to oversee remedial action.

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## Apoptosis

SIR—In reply to B. Kleine (*Nature* 377, 402; 1989), J.F.R. Kerr *et al.* (*Br. J. Cancer* 26, 239–257; 1972) proposed the term “apoptosis” (suggested by Professor J. Cormack, Department of Greek, University of Aberdeen) for a process of active, programmed cell death with a number of characteristic features which clearly distinguish it from “necrosis”, passive cell death. The standard Classical Greek–English dictionary (H. G. Liddell & R. Scott, *A Greek–English Lexicon*, 6th Edn, Oxford University Press; 1869) defines ἀποπτωσις as “a falling off or away”; it is used of the falling of leaves, a clear example of programmed death. The cognate verb is, of course, the reduplicated form ἀποπίπτω; ἀποπτῶω, quoted by B. Kleine, is not known to this dictionary. A computer search of papers published since 1983 revealed fifty with “apoptosis” or “apoptotic” in the title. Had B. Kleine consulted A. H. Wylie *et al.* (*J. Pathol.* 142, 67–77; 1984), cited in our letter (*Nature* 337, 181–184; 1989), rather than his Greek–German dictionary, he would have found a clear exposition of the meaning of apoptosis.

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# Pan-African ornithology divided

T. M. Crowe

A congress that once offered a forum for all African ornithologists has been dealt a crippling blow by politics. African birds and those who study them are the losers.

THE first Pan-African Ornithological Congress (PAOC) was held in Livingstone, Northern Rhodesia (now Zambia) in July 1957. The concept of the congress was the brainchild of a South African, Dr Cecily K. Mackie Niven, who was stimulated to this end chiefly by politically motivated rejections of her invitations, on behalf of the South African Ornithological Society (SAOS), to host the International Ornithological Congress in southern Africa. The first PAOC, with Niven as president, and the next two, were held under the auspices of the SAOS; the editors and many of the officials of those and its two succeeding congresses were South Africans. Dr Gerard J. Morel (chairman of the sixth congress) acknowledged that PAOC is very much a South African invention: "The credit for this unique and enviable situation [the PAOC] is largely to be attributed to our colleagues of the Southern African Ornithological Society".

In 1985, delegates at the sixth congress, held in Francistown, Botswana, voted overwhelmingly for Nairobi, Kenya, as the venue for the seventh congress in August 1988, on the assurance given by Kenyan delegates that a significant number of South Africans would be granted visas to attend. But by 1987 it had become apparent that, for political reasons, South African participation would be at best token. Indeed, for a time there was some doubt as to whether the meeting would take place at all; the Kenyan government officially approved of the congress only a few months before it was due to be held.

Fearing the consequences of a PAOC without contributions from South African ornithologists, many of the members of the PAOC *ad hoc* committee asked the local organizing committee in Nairobi to obtain an unambiguous statement of the Kenyan government's policy on the admission of South African delegates. Some also asked fellow committee members to consider an alternative venue if delegates should be denied visas. Indeed, Nairobi had been abandoned as a venue for two previous congresses on just these grounds.

The response to the first request was vague and non-committal, but it seemed that the Kenyan government would almost certainly not issue visas to South

African passport holders. The response to the second request was that the congress would be held in Nairobi "with or without South Africans". Thus, neither the *ad hoc* nor the local organizing committees considered the presence of South African ornithologists to be essential; prospective participants would have to apply individually for visas and would simply have to "take their chances".

**"One can only speculate as to how much the scientific quality of the congress could have been improved by the contributions of South African delegates, not to mention the thousands of dollars in registration fees that would have been paid by dedicated amateur South African ornithologists."**

In January 1988, those few South African ornithologists who still hoped to attend in August were instructed to provide passport details to the local organizing committee, which would then submit a block application to the Kenyan immigration department. About 15 of us provided these details. But, about three weeks before the start of the congress, I received by courier a batch of visa application forms which had to be filled out individually and returned within that week. On the same day, I received a cable notifying us that our chances of receiving visas had been "weakened by shortage of info from SA". By this time, only six of us still persisted in our determination to attend the meeting.

On the afternoon of 23 August 1988 (five days before the start of the meeting) I was told by telephone by Professor G. M. O. Maloiy, the conference chairman, that my application for a temporary visa had been approved, and that my visa would be in the hands of immigration authorities at Nairobi airport. I was also notified that a similar application for Richard K. Brooke, my colleague at the FitzPatrick Institute, had been approved, but that those of Ian A. W. Macdonald, another institute colleague, Professor Eric H. Harley (of the Department of Chemical Pathology, University of Cape Town) and of Professor Leslie G. Underhill (of the university's Mathematical Statistics Department) and his wife "... were still under consideration". Unfortunately, Brooke and Macdonald had by then cancelled their plans to attend, and Underhill

and his wife were notified by telephone on 26 August (and Macdonald subsequently by post) that their visa applications had been refused. Harley was informed of the success of his visa application on 30 August (two days after the start of the Congress).

Thus, only Harley (holder of a British passport) and myself (a US passport holder) actually attended the seventh congress, as 'official' South African delegates. Dr Richard Liversidge, also a South African resident, had travelled via London on a 'clean' British passport (and thus did not require a visa).

I arrived at Nairobi airport on the evening of 27 August to find that the Kenyan immigration authorities had no record of my visa. Fortunately Dr Hussein Isack, chairman of the local organizing committee, arrived in the nick of time with a photocopy of the visa, and kindly drove me to the hotel into which I was booked. After one evening's stay, I concluded that the hotel was not suitable: two delegates had had their rooms rifled and money and passports stolen; another delegate was brutally mugged nearby. Moreover, the hotel was frequented by aggressive prostitutes and was a costly cab ride from the congress venue, the National Museum of Kenya. Therefore on 28 August I shifted to another hotel which, although it was a mere five-minute walk from the museum, had not been offered as possible alternative accommodation by the local organizing committee.

When I registered that same afternoon I found that my delegate's badge listed the United States as my country of representation (Liversidge and Harley were listed as from the United Kingdom); the country name had been deleted from the abstracts of the two papers I was to present. My packet of congress materials also included a letter from the chairman of the *ad hoc* committee threatening to disrupt the presentation of my papers and to prevent me from attending future congresses if I did not submit manuscripts for the congress proceedings during, or shortly after, the meeting. (I had questioned the hard-and-fast rule in the proposed PAOC constitution that no oral paper could be presented if a complete manuscript was not also delivered.)

Although the seventh congress was



well-attended (more than 100 papers and more than 200 delegates from 19 African and 14 non-African countries), the organization of the programme, the quality of the papers and their relevance to African conservation biology left much to be desired. The original intention had been to have sessions or mini-symposia with themes (seasonality, birds as biological indicators, nest parasitism, pest birds, biogeography and systematics, and so on), many of these were decimated by the enforced withdrawal or exclusion of South African ornithologists. For example, my session on systematics and biogeography had the heart ripped out of it with the absence of Dr Alan C. Kemp (Transvaal Museum), Tony Harris (Transvaal Museum) and Dr W. Stewart Grant (University of the Witwatersrand). I read brief summaries of Kemp's and Grant's papers, but papers delivered *in absentia* have little impact and do not appear in the congress proceedings.

Similarly, the session on birds as indicators of environmental change, which had been organized by Ian Macdonald, was, without him, a ghost of what it could have been. One can only speculate as to how much the scientific quality of the congress could have been improved by the contributions of South African delegates, not to mention the thousands of dollars in registration fees that would have been paid by dedicated amateur South African ornithologists.

On a more positive note, about a quarter of the oral papers were presented by indigenous Africans. But with certain noteworthy exceptions, their presentations were either dry or emotive descriptive accounts or 'how-to-kill-quelea'-type recipes. Few of these papers were based on statistically sound and analysed quantitative information. These deficiencies were certainly not due to the presenters' difficulty with the English language or a lack of dedication, enthusiasm or intellectual ability, but rather can be attributed to poor training in fundamental biological concepts and analytical skills.

### Poor science

Perhaps the most disturbing aspect of the meeting was the poor scientific quality of the conservation papers. Clearly, the main threats to African birds relate to the fragmentation of their habitats (because of man's land-use practices) and concomitant reduction in species' populations. It is simply not good enough to preserve a patch of the preferred habitat of a species if that is too small and isolated to maintain a minimum viable population. Only one paper, by W.D. Newmark, and delegates at a workshop on forest ecosystems convened by Dr Simon Stuart, specifically addressed these issues. At a similar workshop on savannah ecosystems, the convenor was apparently unaware of

the importance of studies of minimum viable population size.

Papers presented during the 'conservation' day devoted to the International Council for Bird Preservation were mainly conservation status-type papers and accounts of educational programmes. There was little or no quantitative biogeography, ecology, genetics or socio-economics. Our job as ornithologists is to provide conservationist organizations' negotiators with the necessary scientific ammunition to sway decision-makers in governments. It is not sufficient merely to make resolutions that this or that chunk of habitat needs to be preserved, but rather to make explicit, scientifically sound predictions about the consequences of its preservation or destruction.

The key clauses in the PAOC constitution that will effectively prevent South African participation in the two remaining congresses this century are the aim to rotate the venues among the four regions of Africa (east, west, central and southern) and the limitation of a single country's membership on the PAOC committee, the ruling body, to a maximum of 3 out of the 20 members. The selection of venues means that the next two congresses will almost certainly be held in central and west Africa. The apportionment of committee members on a strictly regional basis takes no account of geographical variation in the abundance or quality of African ornithologists. Thus, in theory, there could be no South African representation on the committee, but west Africa, which appears now to have no trained resident scientists at PhD level working full-time on birds, will have four members on the committee. When I brought up the issue of the importance of free access to all in choosing a PAOC venue, only one committee member felt that this should even be considered.

One positive development was nevertheless that the committee agreed to consider being indirectly affiliated with the International Council of Scientific Unions. If PAOC adopts the council's code, no venue could be chosen which would exclude delegates on political grounds. But this would not help amateur South African ornithologists, most of whom would still be unable to attend as they do not present papers, the rules applying only to delegates who do present them. This is a particularly tragic blow to African ornithology as nearly half of the delegates attending the first six PAOCs were South African, most of them being dedicated amateurs.

The primary criterion which influenced the choice of the venue for the next PAOC was that it must be in a French-speaking African country. Other factors, such as ease of access (financial and political), excellence of site and excursions or the relevance of birds to local conservation,

were of secondary importance. On this basis Kigali, Rwanda, was chosen as the venue of the eighth PAOC to be held in 1992, with Ghana as a prospective venue for 1996.

### Bitter pill

The PAOC created by Niven is now extinct, and has been replaced by a very different sort of animal. I have reservations about the 'fitness' of this new creature, but will support it as long as there is a reasonable chance of it promoting African ornithology. My message to my South African colleagues is perhaps a bitter pill. As African ornithologists, however, we must endeavour to maintain our tenuous ties with our colleagues to the north. These people need and want our collaboration and we would be remiss if we allowed political differences between governments to sever our ties with them. The virulent anti-South African elements in the PAOC committee are but a comparatively small section of the tail of this animal, powerful enough at the moment to wag the dog, but we have every reason to hope that that power will wane.

At the same time, although the passing of the SAOS-style PAOC is to be lamented, we must continue to promote South African ornithology by sending our best ornithologists, especially young graduates, to discuss their research at international conferences. We must also promote ornithology locally by hosting international conferences in South Africa.

Finally, for too long ornithology in South Africa has been effectively a reserved white profession. This country and its wildlife belong to black as well as white South Africans. As a result of setting and maintaining high standards in our bird research, we have made it very difficult for educationally deprived black people to establish themselves as ornithologists. I believe that we (the SAOS and the FitzPatrick Institute) should do much more to publicize our activities and create incentives to promote the development of black ornithologists, and that we should take active steps to interest black South Africans in conservation biology and ornithology. The University of Cape Town (as well as other open universities) has active academic support programmes which are helping to redress the educational imbalance created by apartheid. □

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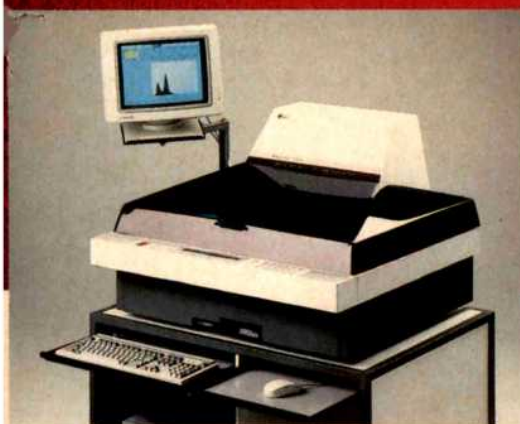
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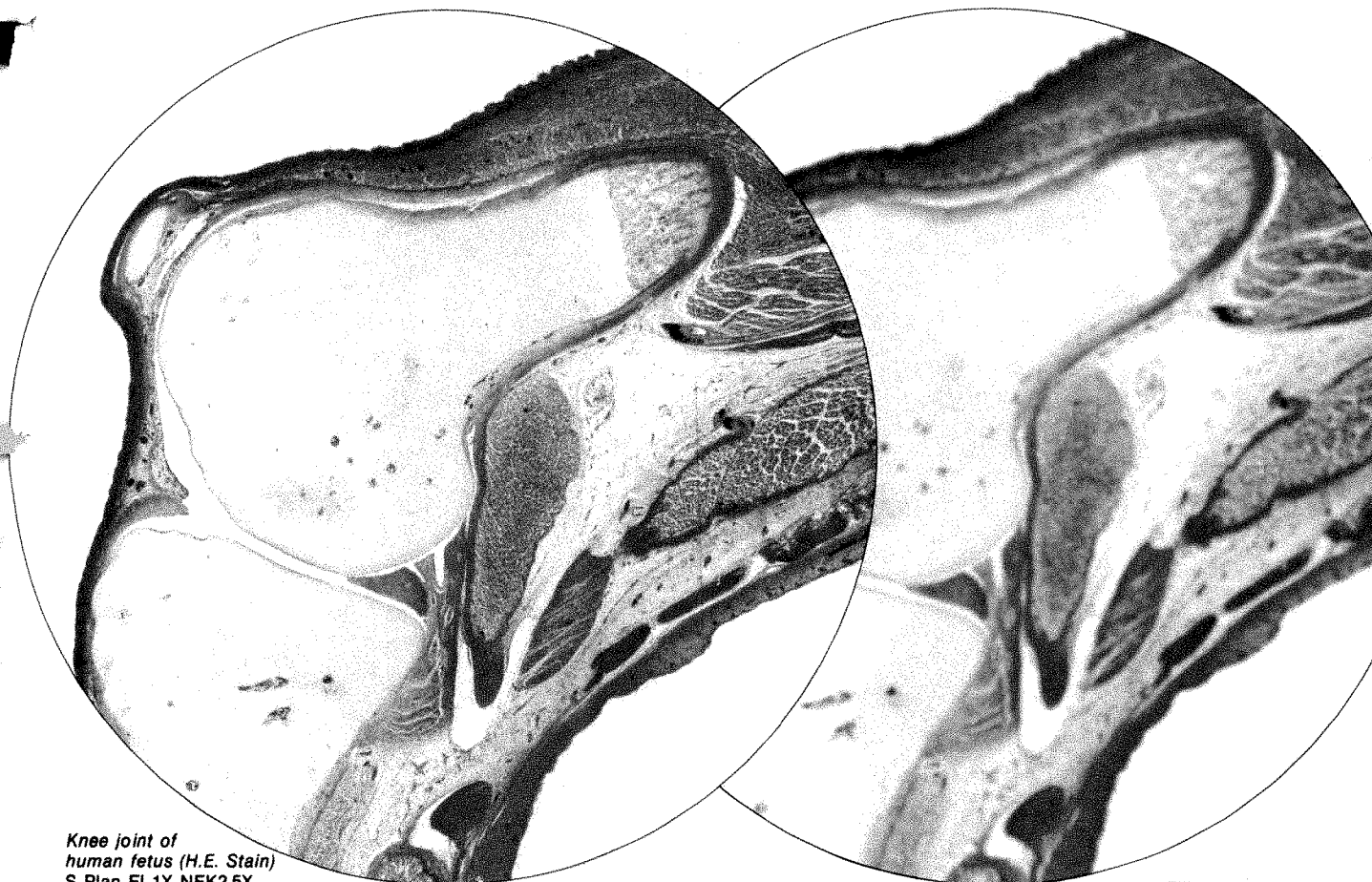
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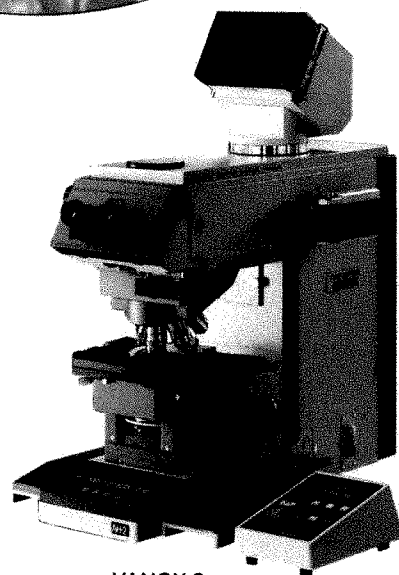
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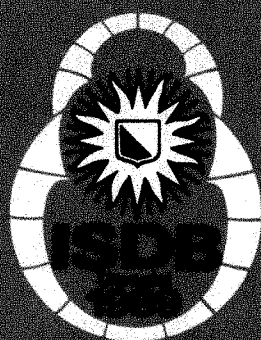
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## FROM DNA TO BODY PLAN - ADVANCES IN THE FIELD OF HUMAN, ANIMAL AND PLANT DEVELOPMENT

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- Groningen August 17-19** "Pathobiology of human germ cell tumors", organisers I. Damjanov, J.W. Oosterhuis, H. Walt. Invited speakers: Andrews, P.W. (Philadelphia), Castedo, S.M.M.J. (Porto), Damjanov, I. (Philadelphia), Engström, W. (Stockholm), Graham, C.F. (Oxford), Pera, M.F. (Oxford), Sikora, K. (London), Skakkebeak, N.E. (Copenhagen).
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# How to say sorry graciously

Mistakes will happen, so that retractions will continue to be necessary. Two physicists have now produced a model of how the task should be tackled.

RETRACTION has become a loaded word, which journals and their contributors alike abhor. As things are now in the United States, there is even a danger that the mere appearance of a retraction will be regarded by a congressional committee or by a self-appointed watchdog as a signal for an inquiry into what has been going on. That is one reason why there should be some rejoicing that it is still possible to publish a full, even a fulsome retraction without bringing down the house on one's head. What has happened is that two physicists at the University of Maryland at College Park (almost in suburban Washington) have retracted the conclusions of an article that appeared last year in no less a journal than *Physical Review Letters* (59, 2507; 1988), but they have done so in such an open fashion that even their sternest critics will be disarmed.

The tenor of the retraction can best be judged from the sentence towards the end in which the authors, O.W. Greenberg and R.N. Mohapatra, say: "We are grateful to Rudolf Haag for warning us of the error of our ways". They then go on to thank another colleague for drawing their attention to an article in the Soviet literature which, if read in time, would also have warned them off. They acknowledge in their retraction (*Phys. Rev. Lett.* 62, 712; 1989) that many people have already embarked on experiments to test their earlier theory. But the best guess seems to be that those who have started down that road will continue, if only because the issue is in itself intrinsically interesting.

The question is whether there can be violations of Pauli's exclusion principle which, applied to electrons, forbids the simultaneous presence of two particles in the same state. An entirely equivalent way of putting this restriction is to say that the complete wavefunction of a system of several electrons (or other particles of the family called fermions) must be anti-symmetric with respect to the exchange of any two of them.

But that formulation has the advantage of suggesting that the wavefunction of a system of several bosons must be symmetric with respect to the exchange of random pairs of particles. The practical consequences are of course considerable. Fermions obey Fermi statistics and bosons (such as photons) obey Bose-Einstein statistics, which explains why the free electrons in a metallic conductor make

only a negligible contribution to its specific heat.

There is a third formulation of Pauli's principle which field theorists find more useful. A particle field, say some arbitrary distribution of radiation (photons), can be conveniently represented with reference to the dynamically possible states of a single photon in the available space. The simplest way of cataloguing the states is by means of the multiply infinite set of states defined by the standing waves which can exist within whatever space is accessible, each of which corresponds to a simple harmonic oscillator whose frequency corresponds to that of the radiation (when the bosons are photons).

Starting from that point and the principle that, in quantum mechanics, observable quantities are operators, the field theorists have fashioned a formalism in which the actual state of a system, which should be a specification of the numbers of particles in each of the possible oscillatory states, can be built up from the interaction of elementary operators, one set for each possible state, which have the effect either of creating or getting rid of a particle from that state. The creation and annihilation operators are the life-blood of field theory.

They are also the simplest means by which departures from Pauli's principle can be handled. For it is clear that the creation operators for fermion and boson fields must have very different properties. Two fermions cannot be in the same state, which means that although the effect of a creation operator on an empty fermion state is to fill it with a single particle, Pauli's principle implies that a second operation on the same state must be a nonsense. But with bosons, the creation operators must plainly behave differently, because there can be many particles in the same state. The algebra that arises by forming the products of several of these operators is simple but intriguing.

Greenberg and Mohapatra's original goal was to study small departures from Pauli's principle. Large departures would not have been surprising in the sense that there is a theory (going back to the 1950s) allowing for particles (which apparently do not exist) with properties intermediate between bosons and fermions, and called parafermions and parabosons. And, of course, they suggested experiments to tell how big the

departures are, or at least to define bounds for them.

Designing experiments is not as difficult as it may seem. If, for example, there is a chance that a single fermion state may occasionally hold two particles, it should be possible to discover that X-rays emerge from conductors carrying electric current, as electrons are occasionally captured into inner energy levels which temporarily violate Pauli's principle. Greenberg and Mohapatra report how some colleagues have completed such an experiment at Fermilab without finding anything untoward.

So where did Greenberg and Mohapatra go astray? Their starting point had been a modification of the algebra of the creation and annihilation operators, which they assumed to be feasible because it had been tried before. What they did not know was that a Soviet researcher, A.B. Govorkov from the international centre at Dubna, had shown as early as 1983 that the mathematical properties of the creation and annihilation operators forbid modifications other than those leading to the uninteresting states of parafermions and parabosons.

One virtue of this retraction is that the reasons why the argument went awry are fully explained, at least in language that those working in the field will readily understand. Another is the good humour of the piece, typified by the acknowledgement of Rudolf Haag "for having shown us the error of our ways". Yet another is the way that those who have helped to put the authors back on the straight and narrow path of rectitude are thanked for their help. And, finally, there are the grant-making agencies: support from the National Science Foundation is acknowledged in exactly the same terms in the two papers.

More than all this, the authors go on to give general reasons why their search for a basis on which Pauli's principle may be violated was a wild-goose chase from the start. The formalisms that give parabosons and parafermions correspond to the orthogonal and unitary symmetry groups of particular integral dimensions, but nobody has yet found a way in which these groups can be generalized into non-integral dimensionality. It seems rather hard on the authors to suggest that their retraction should be taken as a model in this rare genre, but that is what it is.

John Maddox



# Initiation of recombination

J.R.S. Fincham and P. Oliver

RECOMBINATION within genes is generally by conversion — that is, by unilateral transfer of information from one allele to the other. It can be, and often is, accompanied by nearby crossing over, but it is most often the conversion rather than any correlated crossover that is responsible for the recombination within the gene. Much of what is known about this process comes from yeast and other fungi, where conversion shows up as non-mendelian ratios — 3:1 or 1:3 instead of 2:2 — in meiotic tetrads segregating with respect to single mutational differences. The complexity of the alternative models for the mechanism of recombination, although plausible, has hitherto detracted from their testability. Now that Szostak and colleagues have broken into meiotic recombination at the molecular level, as they report in two papers on pages 35 and 87 of this issue<sup>1,2</sup>, we may hope for the emergence of a better verified picture, though no doubt still a complex one.

One of the observations on meiotic conversion that must be explained by hypotheses is that mutational sites often show a gradient of conversion frequency from one end of the gene to the other. This phenomenon, termed conversion polarity, has been taken to suggest that there is a conversion initiation site at one end of the gene and that the probability of a given mutational difference (genetic marker) being involved in conversion decreases with increasing distance from that site.

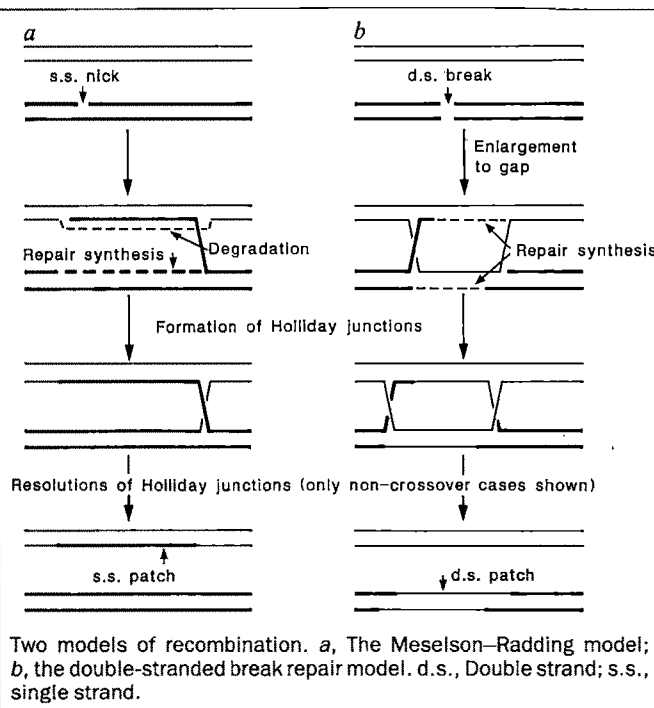
There are two radically different general hypotheses or models of recombination (see figure). Both involve the formation of single-strand exchanges (Holliday junctions) between homologous double-stranded DNA sequences, but they differ in the postulated origin of these junctions. Meselson and Radding's model<sup>3</sup>, which is an adaptation of Holliday's original concept, postulates the assimilation of single-stranded DNA from one duplex into the homologue, displacing the corresponding resident strand starting at a more-or-less fixed point. It is supposed that the displaced strand is destroyed and the single-strand gap in the donor duplex filled by new synthesis. If there is a mutational difference leading to non-complementarity between the invading strand and its new partner, mis-match correction may either restore the recipient to its original sequence or convert it to the sequence of the donor. If no correction

takes place, just one of the two products of the next round of DNA replication will show the conversion (post-meiotic segregation). In this model, the probability of conversion depends on the probability that the genetic marker is included within hybrid DNA which is pictured as extending for a variable distance from the initiation site; hence the closer the marker to the initiation site, the greater the chance of its being involved in conversion. In the original model<sup>4</sup>, the process of single-strand transfer was supposed to be initiated by single-strand nicking in the donor DNA, but another possibility is that it

be the precursor of at least those conversion events associated with post-meiotic segregation, and is now generally accepted as likely to be the immediate precursor of many conversion events. But the Szostak model can also accommodate heteroduplex at the margins of double-strand repair patches. Both invoke a site of preferential cleavage for the initiation of recombination, with the implication that deletion of this site should reduce recombination frequency. But whereas the double-strand gapped duplex in the double-stranded break repair model is necessarily the recipient of genetic information, the single-strand-nicked duplex in the original Meselson–Radding model is the donor. However, in Radding's alternative version of the Meselson–Radding model<sup>4</sup>, it is the single-strand gap in the recipient duplex that initiates conversion.

Both models suggest that the events leading to conversion are also responsible for crossing over, through one kind of resolution of the Holliday junctions. Thus both would give the putative initiation sites an essential role in all recombination, including exchanges of chromosome segments as well as conversion.

In the first of the two papers from Szostak's laboratory in this issue<sup>1</sup>, Nicolas *et al.* present the first evidence at the molecular level concerning the mechanism of conversion polarity. The object of study is the yeast *Saccharomyces cerevisiae* ARG4 gene, in which conversion polarity was demonstrated many years ago by classical genetic methods<sup>6</sup>. First, Nicolas *et al.* introduced various kinds of mutations *in vitro* into defined positions in the cloned gene and, through the now



might be provoked by the appearance of a single-strand gap in the recipient.

The alternative hypothesis of Szostak *et al.*<sup>5</sup>, termed double-stranded break repair, postulates that recombination is initiated by a double-stranded cut that may become a gap by erosion of the cut ends. According to this model, which is strongly supported by experiments on the recombinational behaviour of yeast plasmids containing a double-stranded gap, conversion is the result of repair of the gap by copying from both strands of the undamaged homologue. On this view, polarity could result, at least in part, from variable extents of gapping, with the chance of a marker being included in the gap decreasing with increasing distance from the site of gap initiation.

The two models are not very dissimilar in several of their predictions. The Meselson–Radding model most obviously provides for the heteroduplex which must

routine tricks of yeast molecular genetics, substituted these artificial mutations into the yeast genome. Genetic analysis of crosses between each of these defined mutants and the wild type show a gradient of conversion frequency essentially identical to that previously demonstrated for *arg4* mutations *in vivo*.

In the crucial second phase of their investigation, Nicolas *et al.* made deletions of various segments in and adjacent to the upstream (high-conversion) end of the gene, and tested them for their effects on conversion frequencies of sites at different distances downstream. Deletions covering the region from 30 to 300 bases upstream of the transcription initiation site, when they are present in both parents of a cross, abolish the polarity and reduce the conversion frequency at all sites — not to zero but to a level similar to that which is normally found at the low end of the conversion gradient.

The authors suppose that the main *ARG4* recombination initiation site lies in the -300 to -30 segment, which also happens to include the *ARG4* promoter. When they brought a marker normally at the low end of the conversion gradient close to the promoter by deleting most of the intervening sequence, its conversion frequency was increased to the level characteristic of the high end of the gradient. They attribute the residual conversion activity following the deletion of the putative conversion initiation region to recombination initiated at other, probably more distant sites. When the putative initiation region is deleted in one parent but not in the other, it is the non-deleted partner that is the preferred recipient of information in conversion; there is a large excess of 3:1 over 1:3 ratios of deleted to wild-type products (conversion disparity).

This is, of course, what the double-stranded break repair model would predict. In a substantial proportion of such 3:1 tetrads, a marker several hundred base pairs downstream of the deletion is included in the conversion event, as if the gap extended for at least that distance from the initiation site. Deletions not covering the promoter/initiation region show no conversion disparity, or a disparity not held to be statistically significant (a somewhat questionable conclusion in one case).

In the second paper in this issue from Szostak's group, Sun *et al.*<sup>2</sup> report on the fate, in a diploid culture synchronized in meiosis, of replicating plasmids carrying cloned chromosomal DNA, including the *ARG4* gene, with the putative initiation region either present or deleted. Restriction fragment analysis shows that, when this region is present, three double-strand breaks appear at different sites in the cloned DNA; these are all in, or close to, known promoter regions and one of them (site 2) is in a position consistent with that of the putative *ARG4* recombination initiation site. Each of the breaks affects only a small proportion of the plasmid molecules, perhaps not too different from the observed frequency of gene conversion in *ARG4*. They appear early in meiosis and disappear as meiosis is completed. Plasmids with the putative *ARG4* initiation region deleted are still cut at sites 1 and 3, but not at site 2. These observations provide strong evidence that the recombination initiation site is readily breakable in meiosis and suggests

that a double-strand cut may be the initiating event.

Another observation of considerable interest<sup>2</sup> is that the fragments are substantially shortened by single-strand exonuclease, implying that they have long single-stranded tails. Assuming that these tails are not artefacts of cell disruption, this observation suggests that the gap-

repair process involves the formation of longer tracts of heteroduplex than had previously seemed likely. This would give the double-stranded break repair model still more versatility in explaining the genetic phenomena. □

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## METEOROLOGY

# Cold waters and hot summers

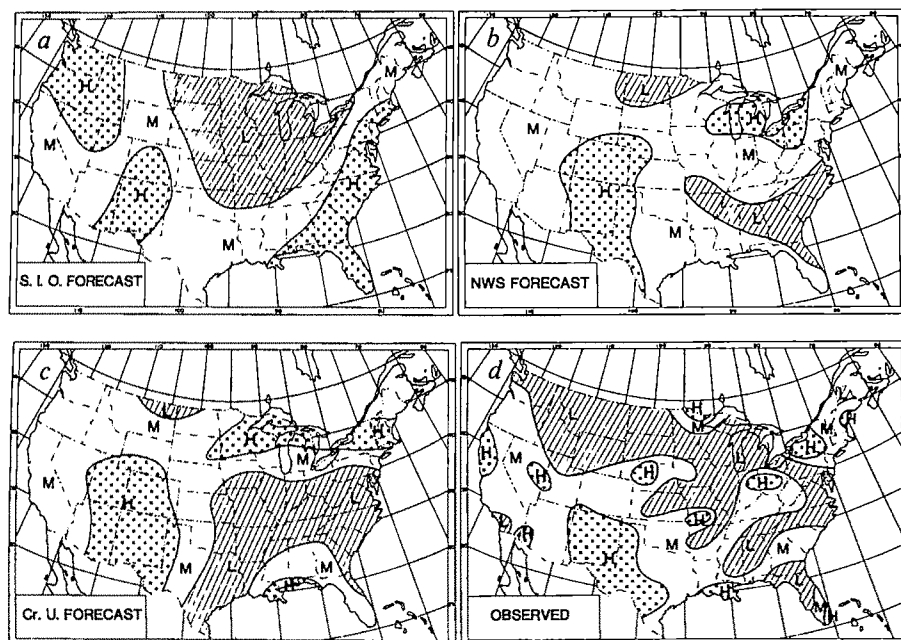
Jerome Namias

THE great US drought of the summer of 1988 was linked to sea-surface-temperature changes, according to Palmer and Branković on page 54 of this issue<sup>1</sup>. Their work extends the recent studies by Trenberth *et al.*<sup>2</sup> who attributed the drought mainly to the arrival of exceptionally cold water in the tropical Pacific (La Niña) in the aftermath of the warm El Niño event of 1987. The novelty of these two contributions is not that abnormal sea-surface-temperature patterns are necessary links in the climate system during the droughts in the United States, but that a surface-temperature anomaly in a specific region is the main culprit.

Empirical studies of many great droughts of this century have demonstrated that summer drought over the United States is part of a larger picture in which three upper-level high-pressure cells — over the North Pacific, the North Atlantic and the United States — interact in a mutually supporting fashion. The term teleconnections has been applied to

such interactions — a phenomenon first explored by Sir Gilbert Walker<sup>3</sup>.

Palmer and Branković<sup>1</sup>, like Trenberth *et al.*<sup>2</sup>, have used state-of-the-art numerical models to make their case. Trenberth *et al.* showed that the three-cell flow pattern at a height corresponding to 300 millibars (in the upper troposphere) is reasonably well simulated if the model is initialized with the abnormally cold sea surface temperature that existed in the tropical North Pacific during the drought. Palmer and Branković compare model global simulations and 30-day weather predictions made starting with the conditions in May 1987 (when no comparable drought existed) with those initialized with the conditions in May 1988. Both papers indicate that sea-surface-temperature boundary conditions were necessary links in the establishment of drought in North America and conclude that the primary cause lay in the tropics where La Niña occurred in 1988. (La Niña is a period characterized by the presence of



Forecasts of (a-c) precipitation classes for summer 1988; a, by myself and D. Cayan; b, the Long Range Prediction Group of the US National Weather Service; and c, Douglas of Creighton University. d, The observed patterns. Classes are terciles computed from 30 summers. L, light; M, moderate; and H, heavy. The models predicted the drought, but not its severity.

1. Nicolas, A., Treco, D., Schultes, N.P. & Szostak, J.W. *Nature* **338**, 35-39 (1989).
2. Sun, H., Treco, D., Schultes, N.P. & Szostak, J.W. *Nature* **338**, 87-90 (1989).
3. Meselson, M. & Radding, C.M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 356-361 (1985).
4. Radding, C.M. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **47**, 821-828 (1982).
5. Szostak, J.W. *et al.* *Cell* **33**, 26-35 (1983).
6. Fogel, E. *et al.* in *The Molecular Biology of the Yeast Saccharomyces* (eds Strathern, J. *et al.*) 289-339 (Cold Spring Harbor, New York, 1981).



anomalously cold surface water in the North Pacific Ocean, and frequently follows El Niño, a period of anomalously warm waters. The cause of these events is unknown, but they have been associated with many types of climate change.)

I believe the conclusion these groups draw is too strong, and argue elsewhere<sup>1</sup> that La Niña is only one of several factors, and by no means the strongest one, associated with the drought through the three-cell structure. Indeed, Trenberth *et al.* and Palmer and Branković acknowledge that this localization of the primary cause is far from conclusively demonstrated by the model results, even though the effect of hemisphere-wide sea-surface-temperature anomalies is strongly suggested. The anomaly patterns are closely related to atmospheric flow patterns, and the cold La Niña water and the warm pool of water just to its north could be either causes of or responses to remote events. Also, Palmer and Branković's data are at best suggestive: there is a disturbing spatial offset between the differences in 300-millibar height patterns and temperature and precipitation patterns over North America and adjacent areas predicted, and those observed.

As both sets of authors point out, the drought of 1988 and its causes could be unique. Many major North American droughts of this century have not been associated with La-Niña-type conditions, although the three-cell pattern has occurred. Dry soil in spring in the mid-west, which generates a high-pressure cell<sup>5</sup>, is conducive to drought (as Trenberth *et al.* acknowledge) and was a factor in 1988. And warm water in either the North Atlantic or North Pacific would also generate high-pressure, anticyclonic cells. It is possible that in the present case, the La Niña event was so strong that it simply eclipsed the other air-sea-land interactions. Thus as is found in other similar studies, cause and effect are hard to distinguish.

The influence of the tropics on atmospheric general circulation, as highlighted by these new studies, is clearly important. Although the drought last year was foreseen in empirical models its severity was underestimated (see figure). Future predictions could benefit from our improved understanding. One thing, however, is absolutely clear: the drought was a consequence of normal atmospheric variability, and has no connection whatever with the greenhouse effect. □

Jerome Namias is at the Scripps Institute of Oceanography, La Jolla, California 92093, USA.

1. Palmer, T.N. & Branković, Č. *Nature* **338**, 54–57 (1989).
2. Trenberth, K.E., Branstator, G.W. & Arkin, P.A. *Science* **242**, 1640–1645 (1988).
3. Walker, G.R. & Bliss, E. *World Weather V. Mem. R. met. Soc.* **4**, 54–84 (1932).
4. Namias, J. *J. Clim.* (in the press).
5. Shulka, J. & Mintz, Y. *Science* **215**, 1498–1500 (1982).

## Detecting density dependence in imaginary worlds

Robert M. May

CHAOS — in the form of simple and fully deterministic systems that exhibit very complicated and effectively unpredictable dynamics — is much in the news, partly, I think, as a result of Gleick's popular book<sup>1</sup>. Although the practical implications of deterministic chaos were first recognized by Lorenz<sup>2</sup> in a meteorological context and by Yorke, Oster, myself and others<sup>3–5</sup> in dynamics of animal populations, most of the recent excitement centres on applications to chemical, physical, electrical and physiological systems, where cascades of period doubling and other related phenomena can be observed in a precise and satisfying way. Among the many reasons for the shift in emphasis is the fact that the magnitudes of real populations of plants and animals are rarely governed by simple nonlinear equations that could provide crisp illustrations of chaotic behaviour. Rather, such populations are typically affected by interactions with many others and by unpredictable environmental fluctuations. The result is a confused picture, with no general agreement about the implications of chaotic phenomena for biological populations<sup>6,7</sup>. A recent paper by Mountford<sup>8</sup> sheds some light on these questions.

One of the uncertainties concerns the extent to which strong nonlinearities and the possibility of chaotic dynamics in natural populations might, in certain circumstances, invalidate conventional methods of analysing data. In the past, the implicit assumption has been that density-dependent effects would regulate a population to some constant value, or at worst to a stable cycle, were it not for unpredictable fluctuations in environmental and demographic parameters. The task thus seemed to be one of extracting a regulatory 'signal' from obscuring 'noise', and conventional techniques (such as *k*-factor analysis, which essentially seeks patterns in the relation between population densities in successive generations) are implicitly based on this. But what if the observed fluctuations in population densities derived partly from real 'noise' and partly from deterministic chaos (apparently random fluctuations produced by a simple density-dependent relation)?

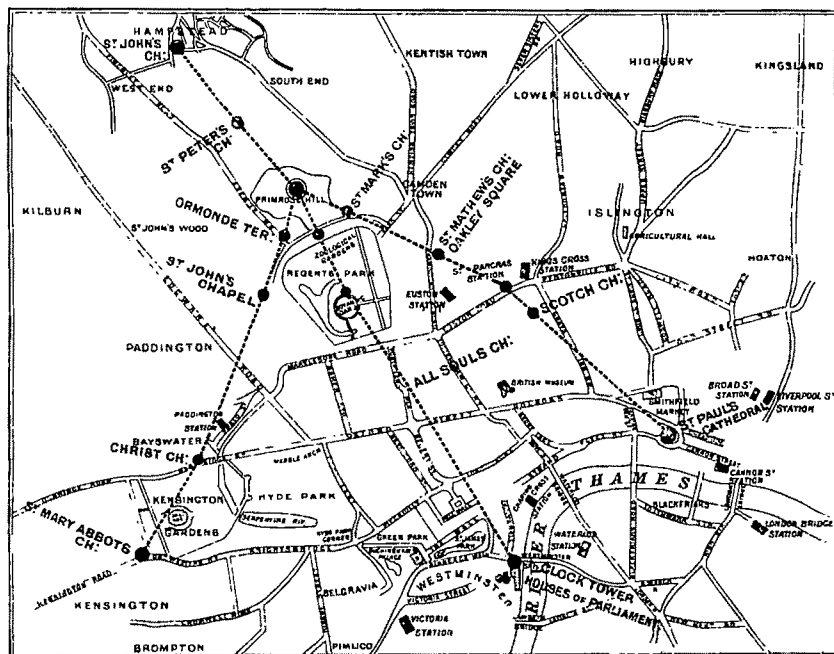
Hassell<sup>9</sup> has attacked this question by examining the extent to which standard techniques of data analysis can uncover the rules obeyed by imaginary populations of his own creation. This general idea is useful in other areas of ecology, for example Colwell and Winkler's exploration of the extent to which prevailing methods could uncover the biogeographical rules

whereby their computer program colonized simulated archipelagos of islands with simulated species<sup>10,11</sup>. Hassell's 'pseudo-data' were generated by a program that embodied ideas about how some insect populations may behave: in each generation adults were distributed (according to a statistical recipe) among many different patches; in each patch, the adults laid eggs; larvae then emerged, and their probability of survival to adulthood depended, in a specified way, on the density in that patch; in this way, the next generation of adults was produced.

Hassell also superimposed various kinds of stochastic fluctuations (in the number of eggs laid by individual adults, for example, or in the patterns of distribution of adults among patches) on his system. If the density-dependent effects acting on larval survival were sufficiently severe, then Hassell's pseudo-data showed irregular fluctuations that were a mixture of the genuine noise he introduced and oscillatory or chaotic fluctuations produced by density-dependent effects. Even when density dependence regulated the population to a constant value in the absence of environmental noise, the stochasticities introduced by Hassell could lead to fluctuations in parameters influencing the severity of density-dependent effects, which in turn could cause the sporadic manifestation of chaotic dynamics.

Applying *k*-factor analysis to these pseudo-data, Hassell could sometimes detect the underlying density-dependent mechanisms that ultimately regulated his populations, and sometimes could not. That is, in this system where the overall density in any one generation depended on a patchy distribution, where density-dependent effects acted differently at different densities in different patches, and where superimposed noise of various kinds made chaos possible in some patches, the regulatory 'signals' were masked by some kinds of noise, but not by others. Hassell *et al.*<sup>12</sup> have now applied these insights to an analysis of about 20 years of data on the abundance of whitefly populations on leaves on viburnum bushes at the Imperial College Field Station at Silwood Park, and seem to have detected density-dependent effects that were not revealed by conventional analysis of the data for overall average whitefly populations in successive generations. But these preliminary studies leave open many questions about which kinds of environmental noise do and do not mask density-dependent signals in Hassell's simulations,

## 100 years ago THE DARKNESS OF LONDON AIR



THE constitution of London fogs has been carefully gone into by several well-known men of science; and the results obtained are of very great interest, as they prove, amongst other things, that during the winter London air has an unusually large amount of carbonic acid in it. Various observations were taken in London during the winter of 1887-88. During the five months selected, Christ Church, Lancaster Gate, and St. Mary Abbot's Church, Kensington, on the south-west line; the Clock Tower, Houses of Parliament, on the south line, and the Scotch Church, Regent's Square; and St. Paul's Cathedral, on the south-east line, were *never once* seen. When it is known that on any fine day during the late spring, summer, and early autumn, you can see right across London, on any one of the selected lines, it will be easy to realize how thick the air over London is during the winter. From *Nature* 39, 442; 7 March 1889.

much less in the real world.

In his new paper<sup>8</sup>, Mountford has extended this discussion. Hassell assumed that the competitive effects determining larval survival are 'scramble': when resources are abundant, all do well, and when resources are sparse, all do badly. The result can be a highly nonlinear, 'boom-and-bust' relation, which can fairly easily produce oscillatory or chaotic dynamics. Mountford, on the other hand, bases all his simulations on density-dependent relations corresponding to 'contest' competition, which assumes resources are distributed in a hierarchical fashion, so that a few individuals do well even in hard times. These smooth relations chosen by Mountford to characterize larval competition can never generate oscillatory, much less chaotic, dynamics. With his pseudo-data generated in this way, Mountford finds that conventional methods can easily detect the density-dependent effects, with spatial heterogeneity and environmental noise presenting no difficulties.

The problems discovered by Hassell seem to me to depend largely on the interplay between what might be called 'density-dependent noise' (generated by non-linear relations that, in some patches in a fluctuating environment, can be severe enough to produce chaos) and 'density-

independent noise' (generated directly by environmental stochasticity), in a patchy world<sup>13</sup>. So I am not surprised that these problems do not arise in systems such as Mountford's, where the nonlinearities are too weak to produce oscillations or chaos. It is perhaps unfortunate that Mountford does not mention this qualitative difference between his chosen density-dependent functions and the boom-and-bust ones of Hassell; the differences between Hassell's and Mountford's simulations seem puzzling if this underlying difference is not appreciated.

This being said, Mountford's study is interesting in several ways. First, it is useful to have an explicit indication that patchiness and stochastic effects, by themselves, are insufficient to upset traditional methods of data analysis. Severe nonlinearities in density-dependent effects thus seem to be an essential ingredient in such problems as may exist. Second, Mountford shows that (in the absence of strong nonlinearities) spatial heterogeneity can actually enhance our ability to detect density dependence: "if there are a number of sets of population density series all with the same mean and the same variability but with differing degrees of spatial heterogeneity, then the detection of density dependence improves with increasing spatial heterogeneity". Third,

the pronounced differences between Hassell's studies (based on 'scramble' competition, with its potential for strong nonlinearities and chaotic dynamics) and Mountford's (based on 'contest' competition) highlight the often-neglected way in which the behaviour of individuals influences the dynamics of populations. Indeed, Lomnicki's recent book<sup>14</sup> is devoted to showing explicitly how some kinds of foraging (or other) behaviour result in 'scrambling' competitive relations and thus in highly volatile dynamics for the population, while different kinds of individual behaviour result in 'contest' competition and thus in tame dynamics. The comparison between Hassell's and Mountford's papers provides another perspective on Lomnicki's basic theme.

There seems to me to be much scope for further studies of ways in which spatial heterogeneity, environmental unpredictability, and nonlinear interactions within and between populations can swirl together to confound empirical studies aimed at understanding what prevents the long-term average density of a population from increasing indefinitely (or decreasing to zero in a time short compared with average extinction times). Using computers to generate pseudo-data for imaginary worlds whose rules are known, and then testing conventional methods of data analysis for their efficiency in revealing these known rules, seems to me to be a useful approach. We must all, of course, join Mountford in agreeing with Dempster and Pollard<sup>15</sup> that "the best hope of unravelling the roles of different factors in the population dynamics of animals, still rests in analysis of long-term, life-table data". The worry remains that, until we have a surer grasp of possible complications in the analysis, we cannot be certain we have gathered the appropriate data, no matter how long and carefully we have toiled in the field. □

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# Big whorls do have little whorls

Ian Stewart

IN 1922 Lewis Fry Richardson wrote *Weather Prediction by Numerical Process*, and it won him election to the Royal Society. For 20 years it was considered disreputable, because of problems with the numerical methods proposed, but by 1955 it had become a classic. It contains a much-quoted parody of Jonathan Swift:

Big whorls have little whorls,  
Which feed on their velocity;  
And little whorls have lesser whorls,  
And so on to viscosity  
(in the molecular sense).

This is the famous Richardson cascade, a descriptive theory of turbulence. Although the mechanism is intuitively attractive, the experimental evidence in its favour has until now been largely indirect. Elsewhere in this issue (*Nature* 338, 51–53; 1989) Argoul *et al.* provide the first experimental geometric evidence for the cascade, and in particular for Benoit Mandelbrot's suggestion that the process is fractal.

If a drop of ink is placed in a jar of water, it creates a vortex ring as it falls. The ring acquires corners, from which smaller vortices break off. The process repeats to form a tree-like structure of

ever-smaller vortices. Each stage is geometrically similar to the previous one, but on a smaller scale — a structure characteristic of fractals. Richardson's idea is that something akin to this proliferating tree of vortices occurs in turbulent fluids. Large vortices break up into smaller vortices — presumably because they are unstable — which themselves break up into still smaller vortices.

The total energy is reduced by viscous friction, but the energy loss is slow to begin with. It becomes much more rapid as the size of vortices decreases, hence Richardson's parenthetical remark. Thus the energy of the fluid cascades into ever-smaller vortices until it is damped out by viscosity.

This suggestion is not Richardson's alone. Similar ideas go back to L. Prandtl, G. I. Taylor, and the statistical theory of turbulence of A. N. Kolmogorov (see A. A. Townsend, *The Structure of Turbulent Shear Flow*; Cambridge University Press, 1976). But how accurate a picture of turbulent flow is it? On physical grounds the idea is fairly plausible; but it has proved extremely difficult to make experimental measurements that are suf-

ficiently precise to justify the model in detail. There is also the difficult problem of extracting the intricacies of vortex dynamics from measurements of a few variables.

Argoul *et al.* overcome many of these difficulties by using a new analytical technique, the wavelet transform. The traditional Fourier transform represents a waveform in terms of a series of sine curves. Instead of sines, the new method represents the waveform in terms of localized solitary waves, more appropriate for nonlinear dynamics. The wavelet transform includes a scaling factor, and can therefore analyse fractal properties. In a sense, it renders visible the construction procedure for the fractal: in this case, the way that a vortex splits into smaller vortices. Successive splittings show up as successive branchings of the graphical representation of the transform.

Argoul *et al.* observe a tree-like branching pattern in high-velocity turbulence, similar to the construction procedure for one of the simplest fractals: the Cantor set. This set is universally misattributed: it seems to have been invented by Henry Smith in 1875, but Cantor drew attention to it in 1883 — citing Smith's work. It is obtained by repeatedly deleting the middle thirds of intervals, and thus has left-right symmetry.

As is made clear in the graphical

## Guanylate cyclases send out new signals

GUANYLATE cyclase has long had to play second fiddle to adenylate cyclase. Both enzymes were discovered in the wake of the identification, 30 years ago, of cyclic AMP as the intracellular mediator of the activation of glycogen metabolism by extracellular adrenaline. Adenylate cyclase and its product, cyclic AMP, have since been established as a much-used system of generating intracellular signals in response to the docking of hormones onto their cell-surface receptors. By comparison, the equivalent system of guanylate cyclase and cyclic GMP is under-employed and, perhaps consequently, often overlooked. But two separate strands of research are focusing renewed attention on the system.

Unlike adenylate cyclase, which is invariably part of the cell membrane, guanylate cyclase can be either membrane-bound or in soluble form in the cell cytoplasm. The membrane form has been intensively studied since the discovery, in the past few years, of the family of atrial natriuretic peptides (ANPs), which use cyclic GMP to mediate their signals that result in the excretion of sodium (natriuresis) and water, and in vasodilation. There has been mounting evidence that the receptor for ANP is, itself, a membrane form of guanylate cyclase. On page 78 of this issue<sup>1</sup>, Michael Chinkers *et al.* provide the ultimate evidence: the complementary DNA

for the enzyme from rat brain, when expressed in cells, has both guanylate cyclase and ANP-binding properties.

The deduced sequence of the enzyme is consistent with the obvious notion that the ANP-binding portion is on the exterior surface of the membrane and the guanylate cyclase on the interior. The sequence of the cyclase portion has considerable similarity with a membrane form of guanylate cyclase that mediates responses of sea-urchin sperm to peptides released by eggs. This observation is hardly surprising, as a complementary DNA for the sperm enzyme<sup>2</sup> was used as the probe to isolate the rat brain complementary DNA.

More interestingly, the sequence of the guanylate cyclase portion of the rat brain membrane enzyme has considerable similarity with the only sequence available for a soluble form of guanylate cyclase<sup>3</sup>, although Chinkers *et al.* conclude that the latter must be the regulatory, rather than the catalytic, subunit of the soluble enzyme.

One striking feature of the soluble enzyme not shared by the membrane form is that it contains a haem group. Some evidence has suggested that it is by interacting with the haem that endothelium-derived relaxing factor, now known from the work of Salvador Moncada and his colleagues<sup>4</sup> to be nitric oxide, activates the soluble guanylate cyclase of smooth

muscle, thereby generating the cyclic GMP that mediates muscular relaxation.

That observation created a flurry of interest and a subsequent broadening of its relevance. It is now known that L-arginine is the endogenous precursor of nitric oxide in endothelial cells<sup>5</sup> and in macrophages<sup>6</sup>, where it is clearly an intermediate in the synthesis of nitrite, but will now also have to be investigated as a possible intracellular signal of macrophage activation by means of guanylate cyclase stimulation. Evidence has also emerged that nitric oxide (not positively identified) mediates the increase of cyclic GMP that follows the interaction of external glutamate with its receptors (of the NMDA type) on the surface of cerebellar cells<sup>8</sup>.

Whether or not guanylate cyclases are destined always to play second fiddle to adenylate cyclases in the events that orchestrate cellular responses to external stimuli, they seem certain to make themselves heard in the coming months.

Peter Newmark

Peter Newmark is Deputy Editor of *Nature*.

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## GEOCHEMISTRY

representation of their results, the Richardson cascade observed by Argoul *et al.* is asymmetric. In other words, during the cascade, each vortex splits into smaller vortices of different sizes. Moreover, the scaling factor does not remain constant throughout the process, so the particular Richardson cascade that they observe is not precisely self-similar.

Recall that every fractal has associated to it a number, its Hausdorff dimension, which measures how irregular it is. Typically this dimension is not a whole number. Introductions to fractal geometry tend to emphasize self-similarity — that apart from the scaling factor the object looks the same at different levels of detail — and define the dimension in terms of constant scaling. But this property is not a prerequisite for being a fractal. In particular the dimension can be defined for fractals that do not have uniform scaling features. On one popular definition a fractal is just a set whose Hausdorff dimension differs from its topological dimension; less technically, it must possess detailed structure on all scales. The simplest way to achieve this is to have the same structure on all scales — that is, to be self-similar — but there exist many fractals whose structure varies from one scale to another. To emphasize this feature, fractals that are not self-similar are often referred to as multi-fractals.

For a rough analogy, modify the Cantor–Smith construction. Instead of always removing the same proportion of the interval, first delete the middle third, then the middle quarters of the remaining subintervals, then middle fifths, sixths, sevenths and so on. Because the deleted proportions vary, there is no true self-similarity, so the modified Cantor–Smith set is a multi-fractal. And to introduce asymmetry, delete intervals that are not centrally placed.

Thus there now exists experimental evidence for the fractal nature of turbulence, clarifying details of the fractal structure, and using this to show that the splitting of vortices is asymmetric and only approximately self-similar. These are features that must therefore be incorporated into any realistic model of the Richardson cascade, or deduced from any more detailed theory of turbulence. These are important results. However, the theory that they confirm is purely phenomenological. The Richardson cascade describes features of turbulent flows — but it does not derive them from the equations of fluid motion. Although fractals are typically associated with chaos in non-linear dynamics — apparently random motion in deterministic systems — these new results bear only indirectly on the relation between turbulence and chaos. □

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# Tales of a lost magma ocean

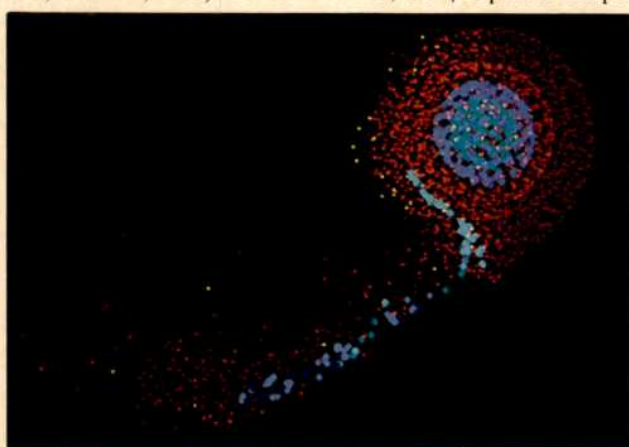
Laura Garwin

CURRENT models for the formation of the terrestrial planets from the dust and gas of the solar nebula postulate a growing population of planetesimals, which in the final stages of accretion may have included about 100 Moon-sized bodies, ranging up to a few the size of Mars. Collisions between these proto-planets have been invoked to explain anomalies in the present-day Solar System, such as the retrograde rotation of Venus, the exceptional tilt of Uranus and the very high density of Mercury (see the recent News and Views article by G. Stewart, *Nature* 335, 496–497; 1988). Closer to home, the

not obvious that this energy will be transferred efficiently to the Earth, or distributed uniformly within it. Jay Melosh (University of Arizona) addressed this question by considering the heating caused by shock waves propagating away from the point of impact. His numerical calculations give temperature rises of 3,000–4,000 K throughout the Earth's mantle for an impactor whose initial velocity  $v$  (at large separation) is 7.8 km s<sup>-1</sup>; for  $v=0$ , at least half the mantle (the hemisphere adjacent to the impact) is heated this strongly. Given a mantle liquidus temperature of about 3,000 K,

even an initially cold Earth would have a completely or substantially molten mantle after the impact.

Independent calculations presented by Al Cameron (Harvard-Smithsonian Center for Astrophysics) yield similar results, and also indicate that the Earth's surface temperature would remain very high for thousands of years, as the rock-vapour atmosphere created by the most intensely heated material (see figure) accreted onto the Earth's surface. The mantle could



Computer simulation of the impact of a Mars-sized body with the Earth, showing the configuration about 80 min after initial contact. Rock particles are in shades of red and yellow, iron particles in blue and green. Lighter shades denote higher internal energy (a measure of the degree of heating or compression following impact).

orbital characteristics of the Earth–Moon system can also be explained by a ‘giant impact’ — in this case, a glancing collision between a Mars-sized body and the proto-Earth, with the Moon forming from the debris of the impact. Elsewhere in this issue (*Nature* 338, 29–34; 1989), H.E. Newsom and S.R. Taylor outline the consequences of such an event for the bulk chemistry of the Earth and Moon, and conclude that the hypothesis is cosmochemically plausible. But given the enormous energy involved in such a collision, might we not expect to see some record of it preserved in the Earth's present structure? A recent conference\* brought impact modellers together with those willing to speculate about conditions on the earliest Earth, to examine the evidence for or against the giant impact hypothesis.

The total energy available from the impact of a Mars-sized body on the proto-Earth is of the order of  $5 \times 10^{31}$  joules — enough to raise the temperature of the entire Earth by about 10,000 K. But it is

remain molten for even longer times, if enough volatiles re-accreted to the Earth to form a dense atmosphere.

The feldspar-rich highland rocks of the Moon are thought to have crystallized from a ‘magma ocean’ more than 100 km deep; if the Earth had its own magma ocean, where are its crystallization products? Unlike the Moon, the Earth is tectonically active, so one would not necessarily expect the products themselves to have survived. But a central tenet of modern geochemistry is that whenever crystals separate from a melt, either during melting or crystallization, a characteristic imprint is left on the chemistry of the melt and crystals. This arises because some elements (the incompatible, or trace, elements) fractionate preferentially into the melt, whereas others prefer the solid phases. A melt can crystallize to a solid of exactly the same composition only if the crystals remain in continuous contact with the melt, so that the chemical system is closed, or if all elements show no preference between crystals and melt (all partition coefficients are unity).

Given the bulk composition of the

\*Conference on the Origin of the Earth, Berkeley, California, 1–3 December 1988.



Earth's mantle, the depth (pressure) and temperature range of the molten layer, and partition coefficients for diagnostic trace elements between the melt and the minerals that would crystallize from it at those pressures and temperatures, it should be straightforward to calculate the effects of a crystallizing magma ocean on the trace-element contents of the minerals involved. This is the approach taken by Ted Ringwood (Australian National University), who has measured high-pressure partition coefficients for various elements, and used them to model the crystallization of magnesium-perovskite from a pyrolite melt. He concludes that a primitive crust would have formed, which was strongly enriched in some elements and relatively depleted in others.

As a result, certain pairs of elements (for example, lutetium and hafnium) would be strongly fractionated relative to one another; yet Ringwood noted that the oldest minerals known ( $4.2 \times 10^9$ -year-old zircons) have chondritic (undifferentiated) Lu/Hf ratios. He concluded that there could not have been a terrestrial magma ocean, unless the Earth was re-homogenized by  $4.2 \times 10^9$  years ago, removing all trace of its existence.

This seeming contradiction between Ringwood's experiments and the existence of a substantially molten early Earth was the subject of much controversy, as other speakers invoked independent evidence for a global magma ocean, or questioned the assumptions and experimental data behind Ringwood's model. Carl Agee (University of Bayreuth) addressed the problem of obtaining a terrestrial upper mantle of peridotitic (olivine-rich) composition from a primordial Earth with the more silica-rich composition of the CI chondrites (primitive meteorites which are thought to represent the composition of the solar nebula). A simple mass-balance argument shows that upper-mantle peridotite can be formed from a CI composition by subtraction of Mg-perovskite and addition of olivine — a combination that can be achieved by flotation of olivine and settling of perovskite crystals in a mantle molten to at least 1,000-km depth. Agee suggested that the observed partition coefficients for elements such as Al, Ti, Hf, Lu, Zr, Sc and Sm were consistent with the 30 per cent fractionation of perovskite required by his mass balance, but this point was hotly contested by Ringwood. Mike Drake (University of Arizona) considered that the addition of 30 per cent olivine would result in substantially non-chondritic ratios of Sc/Sm, Ni/Co and Ir/Au, which are not observed.

There was much debate concerning the reliability of partition coefficients measured at very high pressure — the experiments are difficult to perform, and perhaps even more difficult to interpret —

and about their application to chemical systems more complicated than those of the experiments. But Al Hofmann (Max Planck Institute, Mainz) made the general point that the partition coefficients are bound to deviate from unity, "whether you measure them or not". What must be explained is the general lack of fractionation of trace elements (preservation of chondritic ratios), in the face of a major-element composition so different from chondritic that significant differentiation must have occurred.

In an illustration of the principle that "for each geochemist there exists an equal and opposite geochemist", Herbert Palme (Max Planck Institute, Mainz) pointed out that we do not know that the Earth started out with a CI chondrite composition. He argued that, if the upper mantle is representative of the entire mantle, the Earth's bulk composition is closest to that of the CV chondrites, which have lost silica relative to the CIs, probably in the solar nebula. This proposal avoids the need for extensive differentiation of the Earth's mantle, although there may be problems in detail with CV chondrite as a parent material for the Earth.

In contrast, Don Anderson (California Institute of Technology) put his faith in "seismic chemistry" as a more reliable guide to the composition of the bulk mantle than samples of upper-mantle

peridotite. He cited density and seismic-velocity data supporting an FeO content of about 14 per cent for the lower mantle, as compared with values of 8–12 per cent for the upper mantle. Although this FeO content is high compared to chondrites, it is comparable to a recent measurement for the solar photosphere (H.H. Breneman & E.C. Stone *Astrophys. J.* **289**, L57; 1985), and to the values inferred for the Moon and Mars. So this line of argument forces us once again to explain the Mg-rich upper mantle by large-scale chemical differentiation, and to confront the paradox of unfractionated trace elements.

Returning to the question that prompted the conference, it seems that mantle geochemistry cannot (yet?) be used to support or contradict the giant impact hypothesis. As Anderson emphasized, Ringwood's experiments (or, more generally, partition coefficients substantially different from unity) do not rule out melting; they only rule out fractionation at mantle pressures. Thus, if crystals could be kept in contact with the melt throughout crystallization, for example by entrainment in a turbulently convecting flow (W. B. Tonks, University of Arizona), a global magma ocean could come and go without leaving any imprint on the chemistry of the mantle. □

Laura Garwin is Physical Sciences Editor of Nature.

## POLYMERS

# Surface segregation of blends

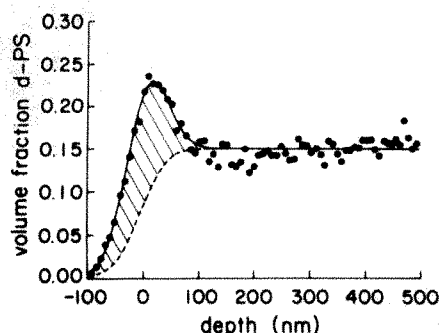
A. M. Donald

Ask physicists what length scale they associate with surface phenomena and they are likely to come up with a value of 1 nanometre or less. But for polymers, because of their large chain length, the distance associated with a surface 'monolayer' is much larger, and corresponding timescales much longer than for simple atomic or molecular systems. Thus polymer systems could be ideal for experimental tests of recent theoretical predictions concerning wetting and surface phase transitions in binary mixtures, which relate directly to the precise composition of the surface. Few experiments have yet been carried out to look at the surface composition of polymer blends (miscible two-component systems) but new results from E. J. Kramer and colleagues<sup>1,2</sup> indicate that a very marginal difference between the surface energy of polystyrene and its deuterated form gives rise to a dramatic enrichment of the latter at the surface relative to the bulk composition. It is to be expected that even more striking effects could be seen with blends containing chemically distinct polymers.

Few polymer pairs are miscible. This can be explained in terms of the small

entropy of mixing relative to the total entropy: the number of configurations in a long chain system is already so large there is little to be gained by mixing in a second species. Thus mixing will occur only when there is a sufficiently favourable specific (enthalpic) attraction between the two components. A particularly simple blend system arises when the two components differ only by isotopic substitution, for example of all the hydrogen atoms (<sup>1</sup>H) on the chain by deuterium (<sup>2</sup>H). Polystyrene blends become miscible above a critical temperature (176 °C for the molecular weights used in the study of Kramer and co-workers), and thus in the bulk there is a homogeneous distribution of the two components at temperatures greater than this.

But what happens in the vicinity of a surface of a miscible blend? If the two components have different surface energies, then a driving force exists to permit the component of lower surface energy to segregate preferentially at the surface — as is known to occur for binary alloys and liquids of small molecules. The technique that Kramer and co-workers have used, forward recoil spectrometry



Volume fraction of deuterated polystyrene (d-PS) blended with polystyrene as a function of depth, after annealing at 184 °C for 5 days (initial volume fraction 0.15). Shaded area, surface excess. (From ref. 1.)

(FRES), is well-suited to tackle this problem for isotopic blends, because it permits the relative amounts of  $^1\text{H}$  and  $^2\text{H}$  to be measured as a function of depth.

In the technique, high-energy (about 3-MeV) helium ions are incident on the sample at a glancing angle. When these ions collide elastically with the  $^1\text{H}$  and  $^2\text{H}$  nuclei, the nuclei are ejected and subsequently detected; a foil screens the scattered  $\text{He}^{2+}$  ions from the detector. Kinematic theory predicts the energy with which nuclei at the surface are ejected (the energy separation between the two types of nuclei is about 0.5 MeV). For nuclei below the surface, energy is lost owing to frequent collisions with electrons, and so the peaks associated with the two types of ions are spread to lower energies, with a peak shape determined by the composition profile. The depth resolution of the technique (about 80 nm) is largely limited by the straggling in the stopper foil.

This technique was originally developed to look at  $^1\text{H}$  and  $^2\text{H}$  profiles in metal<sup>1</sup>. More recently Kramer's group have extended it to look at various diffusion problems in isotopic polymer blends. For the case of preferential segregation of one component at a free surface, the resolution is insufficient to reveal the detailed structure, but Kramer and colleagues can observe a marked enhancement in the average concentration of deuterated polystyrene relative to the bulk concentration (see figure). From the dependence of the surface excess on the bulk composition, the authors calculate, using an analysis developed by Schmidt and Binder<sup>4</sup>, that the difference between the surface energy of the protonated and deuterated forms of polystyrene is a mere  $8 \times 10^{-5} \text{ J m}^{-2}$  — far too small to be measured directly.

But clearly, if such a weak effect in polystyrene (which arises from differences in polarizability between  $\text{C}-^1\text{H}$  and  $\text{C}-^2\text{H}$  bonds) leads to such a strong surface enhancement, then for polymer blends of practical importance, which typically contain components with very different chemical properties, the surface enhancement should be striking.

Because it is the blend's surface which

determines many of its properties — environmental resistance, wetting and friction, for instance — this finding is not simply of theoretical interest. In addition the surface properties may vary with the route of blend preparation — that is, whether this surface enrichment has time to become established.

The FRES technique is currently being refined by Kramer and co-workers, who are looking at ways the use of a stopper foil can be avoided to improve the resolution. They now claim<sup>2</sup> to have a detector with resolution better than 35 nm — capable of examining the composition profile in considerable detail. Other surface techniques with high spatial resolution are also becoming available for polymer systems. These include secondary-ion mass spectroscopy and X-ray photoelectron spectroscopy, but the one that looks most promising and is attracting most attention is neutron critical reflectometry. Many laboratories, including the Rutherford-Appleton, are now setting up

appropriate spectrometers (see ref. 5 for the first application to a polymer interface). Although this technique cannot provide such a direct measure of chemical composition as FRES, its high resolution (estimated to be about 5 Å) will permit checking of any hypothesized distribution of components. In this sense it can be regarded as complementary to FRES. A burst of activity in the field of polymer surfaces therefore seems likely, with implications for an improved understanding of surface phenomena at both the pure and applied levels. □

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#### NATURAL SELECTION

## Why do plants produce so many more ovules than seeds?

Deborah Charlesworth

MANY flowering plants produce far fewer mature fruits than flowers, and fruits often contain fewer seeds than the number of ovules that were available for fertilization. A striking example of a plant in which there is a low value of the number of seeds produced compared with the number of available ovules is the shrub *Dedeckera eurekaensis* described by Wiens *et al.* on page 65 of this issue<sup>1</sup>.

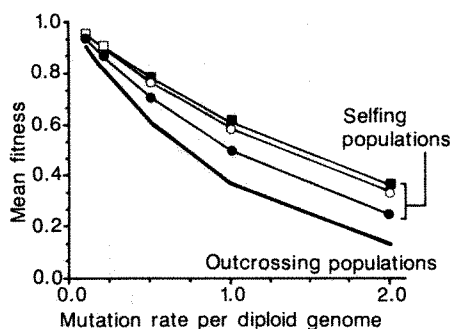
One obvious possibility, which seems to be at most only part of the story, is that flowers sometimes do not get enough pollen to fertilize all the ovules. The supply of pollen (or uncertainty of its receipt) can often limit the chance that a flower sets a full complement of seeds<sup>2,3</sup>. In self-incompatible species, pollinators may deposit the plant's own pollen on its stigmas, perhaps clogging up the flowers' stigmas so that they cannot later be fertilized<sup>4,5</sup>. Similarly, pollen from the wrong species might reduce flowers' chance of fertilization. In self-compatible species, ovules fertilized by self pollen may yield inviable seeds so that seed production in nature may be low. P 36,657

In many cases, however, including that of *D. eurekaensis* described in this issue<sup>1</sup>, pollen supply does not seem to be the main limiting factor for fruit set<sup>6</sup> or seed set per fruit<sup>7</sup>. Because it is also frequently found that many fruits and seeds initiated are aborted even when plenty of compatible pollen is deposited on the stigmas<sup>8–10</sup>, the role of the 'excess' flowers and ovules

cannot be to increase female fertility, and fruit abortion probably indicates limitations in the amount of resource available for the (presumably) costly process of fruit maturation<sup>11</sup>. Under natural pollination conditions, outcrossing species generally have lower seed/ovule and fruit/flower ratios than do inbreeders, and so do long-lived woody species<sup>9,12,13</sup>. The existence of these clear between-species differences suggests that these ratios are probably not just consequences of the local environmental circumstances in which the particular plants studied were growing. Interpreting such comparative data is difficult<sup>14,15</sup>, in this case especially so because the characteristics of the different species are correlated: for example, annual species include a much lower fraction of outcrossers than do perennials<sup>13</sup>. Thus the low ratios of perennials might result from their more outbreeding population structure, compared with annuals, rather than from their living longer. The patterns seen can be greatly clarified by experimental data, which can help in the assessment of the contributions of the two most plausible types of explanation.

The first explanation is based on the idea from the theory of life-history evolution that perennial species must allocate resources between the competing functions of pollen and ovule production at the time of flowering (which probably determine later expenditures necessary to mature the fruits) and storage of nutrients





Mean fitness of a completely outcrossing population at its equilibrium under mutation to deleterious partially recessive alleles at many loci, compared with the corresponding mean fitness of populations reproducing only by self-fertilization, with different mutation rates per diploid genome. The outcrossing mean fitness does not depend on the type of selection or the values of the selection parameters, so only a single line is shown. For the selfing cases, results for various values of the selection coefficient of a mutant allele ( $s$ ), and the dominance coefficient ( $h$ ) are: black circles,  $s=0.5$ ,  $h=0.5$ ; open circles,  $s=0.2$ ,  $h=0.2$ ; squares,  $s=0.01$ ,  $h=0.2$ .

for growth and survival to the next season; they are therefore expected to have a low rate of reproduction and thus produce few seeds per unit mass of plant in any one season<sup>16</sup>. Why then do they have many flowers and ovules? This can be accounted for in terms of the male fertility component of fitness through pollinator attraction<sup>17,18</sup>. It is then surprising that plants do not frequently produce some flowers with purely male function and become andromonoecious. Total loss of female function of flowers may be disadvantageous because of uncertainty of the pollination process; unless each flower has a high chance of getting fertilized, or unless fruits are very costly of resources (for example very big), maximum-realized fertility cannot be achieved from a few flowers<sup>19,20</sup>.

Another interpretation is that outcrossers suffer high genetic loads, causing many fertilization products to abort early in development<sup>21</sup>. (Genetic load is the relative lowering of the mean fitness of a population compared with the fitness of the best possible genotype.) Genetic loads are thought to result mainly from mutation to harmful partially or fully recessive alleles such as recessive lethals, and possibly also to alleles maintained by heterozygote advantage<sup>22</sup>. The second type of genetic load is least with outcrossing and increases somewhat with self fertilization (selfing), presumably because heterozygote advantage helps maintain variation in populations even though homozygotes produced by selfing are at a disadvantage. This type of gene action cannot therefore explain the low fecundity of outcrossing species.

With genetic load resulting from mutation, mean fitness increases as selfing increases because the equilibrium fre-

quences of deleterious alleles are reduced. The total genetic load in a wholly selfing population can be much lower than in a completely outcrossing population when the mutation rate is high, so that individuals in the outcrossing population are heterozygous for many mutant alleles depressing fitness (see figure). It seems unlikely that all this load will be expressed in the earliest stages of development so as to be detectable as reduced seed maturation, but this view might be wrong, as this is an important developmental stage. It is not known what proportion of plant genomes are expressed in the seed stage; certainly many mutations are known that affect seed (embryo and endosperm) characters<sup>23,24</sup>.

If mutational load contributes to the loss of fertilization products in outcrossing plants, the abortion of individual seeds will be determined by the zygote genotypes; it should be random within fruits, and its frequency should be affected by the maternal and paternal parents, and by interaction between them<sup>25</sup>. Furthermore, in outcrossing populations with mutational load including partially or fully recessive alleles, strong inbreeding depression would be expected; in *D. eur-ekensis* Wiens *et al.* find that outcrossed flowers produce more mature seeds than selfed ones, but the difference is not statistically significant. This hypothesis could also account for the correlation between longevity and low female fecundity if long-lived species have higher mutation rates, as has recently been argued<sup>26</sup>. □

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## Surprising damp

DAEDALUS once devised an anti-condensation paint for kitchens and bathrooms. It was based on the 'hydrogel' polymers used for soft contact lenses; these readily absorb water, swelling somewhat as they do so. A hydrogel paint can thus absorb sudden peaks of condensation, and slowly breathe it out again as vapour in periods of low humidity. With the walls and ceiling of the whole room as an absorption surface, many litres of condensation can be taken up.

The problems began when Daedalus tried to colour his new product for the domestic market, by mixing it with a rather hastily formulated emulsion paint. In a cold, damp room the new mixed paint certainly took up water vapour very efficiently. But when the room was warmed up to reduce its relative humidity, condensation actually oozed from the painted surface and ran depressingly down the walls. It turned out that the liquid emulsion droplets dispersed in the paint had solidified in the cold test room. On warming up, they melted again, expanding strongly as melting vegetable oils do. The water-swollen hydrogel was thus suddenly and forcefully pressurized from within, and its loading of liquid water was squeezed out of it by reverse osmosis. Daedalus had invented a condensation generator.

DREADCO's marketing department soon rose to the challenge of this new product. Wet Paint® is being aimed at the car and vehicle market. The weekly wash and polish beloved by devout car-owners is mainly symbolic: corrosion starts at internal seams and surfaces no owner can reach. But a Wet-Painted car will never need to be cleaned at all. Every night, as the temperature falls and humidity rises, its surface will absorb water vapour; every day, as the temperature rises, it will weep the water out again as a liquid. Coming out of the paint surface itself, the exuded water will clean far more efficiently than any external spray. Dirt and road salt will simply be dislodged from beneath and floated away. Applied to trains, houses, bridges, and many other external structures, Wet Paint will keep grime, mould and graffiti at bay. The whole urban environment will be wonderfully smartened up.

Some technical problems remain to be solved, of course. During its pressure-exudation phase, Wet Paint tends to weep not only at its outer surface, but also at its inner one, thus pumping itself forcefully off the wall. Reliable bonding to substrate or undercoat is needed. On the other hand, DREADCO's cosmeticists hope to apply this principle in a new and powerful moisturizing cream. Unique among such preparations, it will take up moisture from the air and force it into the wearer's skin under many atmospheres of hydraulic pressure.

David Jones

# The pulsar inside SN1987A

SIR—The discovery<sup>1</sup> of a pulsar inside the remnant of supernova 1987A is not in itself surprising. The initial neutrino burst<sup>2,3</sup> suggested the formation of a neutron star, and the low-energy X-ray flux (6–16 keV) observed by Ginga<sup>4</sup> has been interpreted as evidence for an active pulsar. Apart from fluctuations on time-scales of days, the soft X-ray flux has remained constant at about  $6 \times 10^{36}$  erg s<sup>-1</sup> since its first appearance in January 1988 (Y. Tanaka, personal communication). The harder emission, above 20 keV, has declined in the past few months<sup>5</sup>, in accordance with the radioactivity model<sup>6</sup>.

We have suggested that the soft X-ray flux could be synchrotron emission from a non-thermal nebula, fed by the remnant pulsar at a rate of about  $10^{38}$  erg s<sup>-1</sup> in the form of relativistic particles and magnetic field<sup>7,8</sup>. The emission could be detected if the supernova envelope had undergone early fragmentation, shortly after the explosion. The pulsar input would amount to a significant fraction of the present bolometric luminosity of the supernova, and deviations in the decay of the light curve should be expected: preliminary indications of an extra energy input may already be available<sup>8</sup>.

The finding of the pulsar lends strong support to these ideas. The properties of the pulsar, however, are rather surprising, especially its extremely short period and the small value of the surface magnetic field.

The strength of the magnetic field at the light cylinder radius,  $\sim 2.4 \times 10^9$  cm, where field lines corotate with the pulsar would move tangentially at the speed of light, is bracketed by the requirements that the electromagnetic energy output be less than SN1987A's present bolometric luminosity,  $2.1 \times 10^{38}$  erg s<sup>-1</sup>, and more than the pulsed optical luminosity,  $6.4 \times 10^{35}$  erg s<sup>-1</sup>. Thus  $2.3 \times 10^9$  G  $< B < 4.2 \times 10^7$  G, corresponding to a surface field between  $3.3 \times 10^7$  and  $6 \times 10^8$  G if dipolar geometry is assumed.

The SN1987A pulsar thus belongs to the class of weakly magnetized, rapidly rotating objects for which a 'recycling'

scenario is accepted as canonical. Clearly, we have here first-hand evidence that at least some objects of the class are 'primordial'<sup>9</sup>. One may then wonder about the size of the millisecond pulsar population, because a new production mechanism has been found and since the radio surveys performed so far have not covered adequately the submillisecond range. Also, we note that, if pulsars such as this one are born frequently, their magnetic field cannot be amplified up to  $10^{15}$  G later on by thermoelectric instabilities<sup>11</sup>, because this would entail a huge, delayed release of rotational energy, for which we have no astrophysical evidence.

In the discovery observations a weak sinusoidal modulation of the pulsar frequency is noted. If confirmed and interpreted at face value as evidence for a binary companion, the modulation would imply a companion mass of  $\sim 10^{-3}$  times the mass of the Sun. At any rate, whether the claimed modulation is real or only an upper limit, the interpretation that the soft X-rays from SN1987A result from accretion<sup>12</sup> appears unlikely.

Concerning the nature of the optical radiation mechanism, we note that the brightness temperature of the observed pulses, even including the entire speed-of-light surface, corresponds to an electron Lorentz factor of at least  $1.6 \times 10^4$ , whereas the Lorentz frequency in the minimum magnetic field is already  $6 \times 10^{12}$  Hz. Then, in order to satisfy the thermodynamical constraint, one has to invoke either synchrotron radiation by protons, or by coherent processes, or, perhaps, electron synchrotron radiation well beyond the speed-of-light distance.

Another puzzle concerns the variations observed in the average pulsar optical luminosity. One could imagine a single physical cause for them and for the 8-h period of the frequency modulation, if the latter is confirmed. More likely, they could be attributed to the effect of the inhomogeneous nebular medium, in the same way as postulated for the fluctuations of the soft X-ray flux<sup>7,8</sup>. Although homogeneous models of the supernova envelope become transparent to optical radiation a few months after the explosion<sup>13</sup>, the optical spectra of SN1987A still show clear signs of opacity effects, probably due to bound-bound and bound-free transitions of metals (E. Oliva, personal communication); if the envelope material is clumpy, and if the clumps have some non-radial motion, temporary occultations of the pulsar are a reasonable possibility.

Finally, we recall that a rotation frequency of 2 kHz is close to the hard limit of the virial theorem, and strains the predictions of certain stellar interior models on the onset of non-axisymmetric

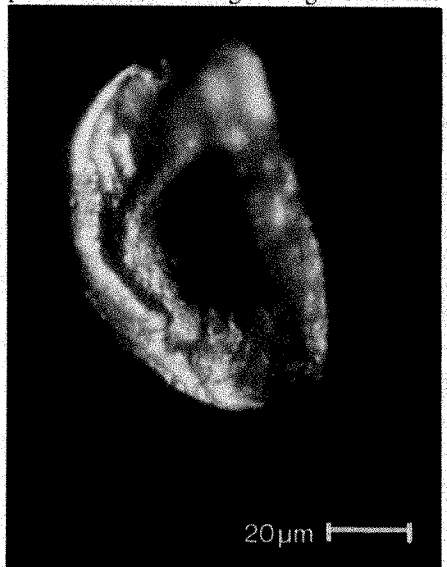
instabilities<sup>14</sup>. The mere fact that such a rotation is observed after two years implies a mass quadrupole moment smaller than  $5.3 \times 10^{40}$  g cm<sup>2</sup>. The above limit corresponds to a gravitational wave luminosity of  $6.1 \times 10^{14}$  erg s<sup>-1</sup>, which still would be the main energy output channel by a very large margin. A measurement of the first and second derivatives of the period would establish the relative importance of gravitational radiation losses.

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## Hair light guide

SIR—During studies on the staining of whole plucked hairs for use in biological dosimetry, we have observed that grey hairs illuminated at one end emit light at the other (J. Wells, *Stain Technol.* **63**, 189; 1988). An example of this phenomenon is illustrated in the figure. A 6-mm-long grey hair was mounted through a piece of card, ensuring that light could not



pass between the card and hair, and the card was placed on a microscope stage and illuminated from below. Of the 2 mm of hair protruding through the card, only the cut end emitted sufficient light to be seen down the microscope. Reduction in light intensity was noted with increased hair length but by far the most important factor was hair colour; little or no light was transmitted by brown hair.

Thus, grey hair acts as a natural fibre optic which can transmit light to its matrix, the follicular epithelium and to the dermis. Whether light transmission down hairs affects skin and hair needs investigation.

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## DNA helical repeats

SIR—The recent paper, "The helical repeat of double-stranded DNA varies as a function of catenation and supercoiling", by Wasserman *et al.*<sup>1</sup> gives the misleading impression that the authors have solved the fundamental problem of the partition function between twist and writhe in supercoiled DNA. In fact, the authors did not measure the DNA helical repeat,  $H$  (ref. 2), but a newly defined parameter,  $h$  (ref. 3).

Whereas  $H$  is uniquely determined by the three-dimensional structure of a given DNA molecule,  $h$  measures the relationship between the DNA and a chosen reference surface<sup>3</sup>, and is therefore related to DNA structure only indirectly. Thus, the differences in  $h$  calculated by Wasserman *et al.* correspond to changes in the relationship between the DNA and a reference surface rather than to changes in the internal structure of the DNA. The results of Wasserman *et al.* therefore have no relevance to the energetic constraints on DNA deformation. Furthermore, although the parameter  $h$  has a clear biological meaning when it relates DNA to a real surface, its significance becomes doubtful when the surface is only imaginary.

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COZZARELLI *ET AL.* REPLY—For a DNA whose axis lies on a surface, real or virtual, there are two related measures of the turning of the double helix: twist ( $Tw$ ), which measures a component of the rotation of one strand about the axis, and winding number ( $\Phi$ )<sup>3</sup>, which is the sum of the periodic exposures of either strand away from the surface. The helical repeat of a DNA with  $N$  base pairs has been defined in two different ways, namely  $(N/\Phi)$  and  $(N/Tw)$  or  $h$  and  $H$ , respectively, in the nomenclature of Stasiak *et al.* Both are valuable measures of DNA structure and differ only when DNA is supercoiled.

Contrary to the assertion of Stasiak *et al.* however,  $h$  is often the property of biological significance. It is also much more easily measured. The parameter  $h$  defines the sequence periodicity of nucleosomal DNA (ref. 4) and the phasing of protein-binding sites, and is, in fact, most commonly measured by the accessibility of DNA to chemical or enzymatic probes. In our experiments,  $(N/\Phi)$  not

$(N/Tw)$  is determined by the catenane-induced supercoiling<sup>1</sup>. Supercoiling derived from catenation, linking number deficit, and the wrapping of DNA around histones, in all cases, leads to a change in  $h$  (and also  $H$ ); it is thus obviously incorrect for Stasiak *et al.* to assert that  $h$  has no relevance to DNA structure or deformation. Stasiak *et al.* make an additional error; Wang<sup>2</sup> did not distinguish  $H$  from  $h$  as he used relaxed DNA, for which the two are equal.

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## Cystic fibrosis

SIR—Kitzis *et al.*<sup>1</sup> confirm preliminary reports<sup>2</sup> of a segregation distortion of cystic fibrosis (CF) alleles with the sex of carriers. They use informative linked DNA markers to track CF alleles in families where there are carriers of the disease and count the numbers of carriers and non-carriers amongst unaffected siblings. The male/female ratio is as expected (1/1) and the carrier/non-carrier ratio follows mendelian expectation (2/1). But the male/female ratio for CF carriers is 1.21/1.0 (with a complementary 1.36/1 female/male ratio for non-carriers). We bring to your attention work reported by Gedschold *et al.*<sup>3</sup>, which we believe indirectly supports this segregation distortion phenomenon. These authors analysed questionnaires from a population-genetic study<sup>4</sup>, to show that for East German CF families, female carriers had more sibs (1.99 on average) than male carriers (1.66).

These two findings are remarkably consistent; the segregation distortion will generally lead to female carriers being found in larger sibships than male carriers. Using a male/female carrier ratio of 1.21/1.0, the likelihood ratio of families ascertained through one female carrier with 1.99 unspecified sibs producing  $n$  carrier sibs to families with one male carrier and 1.66 unspecified sibs producing  $n$  carrier sibs is almost unity (that is, ignoring constant terms,  $1.21 \times 1.66/1.0 \times 1.99 = 1.0093$ ).

Hence, we conclude that the segregation distortion of CF alleles with sex explains the differences in sibship sizes for male and female carriers. Linkage dis-

equilibrium between the CF allele and alleles detected by DNA probes can be used to modify prior risks for individuals seeking carrier determination/exclusion<sup>5</sup>. Perhaps, now that the segregation distortion is confirmed, risk calculations should include the consultant's sex.

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## Unhealthy genes

SIR—In a recent review article (*Nature* **336**, 435; 1988) Kondrashov asks whether future generations will gradually have less healthy genes. I found his article particularly interesting as I have recently started a debate in Norway concerning this problem.

The rate of mutation of human genes is no less than before. In industrialized societies, where the combination of medical care, lifestyles and reproduction strategies have decreased the selection for robust individuals, unwanted mutations will therefore be expected to accumulate.

The known genetic diseases represent only a small part of this problem, simply because they represent only a few specific mutations in a very limited number of genes. We have about 100,000 different genes (plus a large amount of DNA that influences gene activity) and each gene can mutate in many different ways. Some mutations are lethal, and therefore easily selected against, but most are probably either relatively neutral or slightly detrimental and do not cause any overt disease that can be traced to a specific mutation.

As a consequence we must, in the long run, expect an increased frequency of various health problems, implying that an increasing fraction of resources will need to be directed to health care.

I believe that an effort should be launched to evaluate the problem. The most important question is how fast the deterioration takes place. Depending on the outcome of this evaluation, it may be worth investigating possible means of dealing with the problem.

Kondrashov points out that the increase in unhealthy genes is a practically irreversible process. The irreversibility lies in the fact that we are dealing with humans and not animals, for which relevant selection programmes would be easy to implement.

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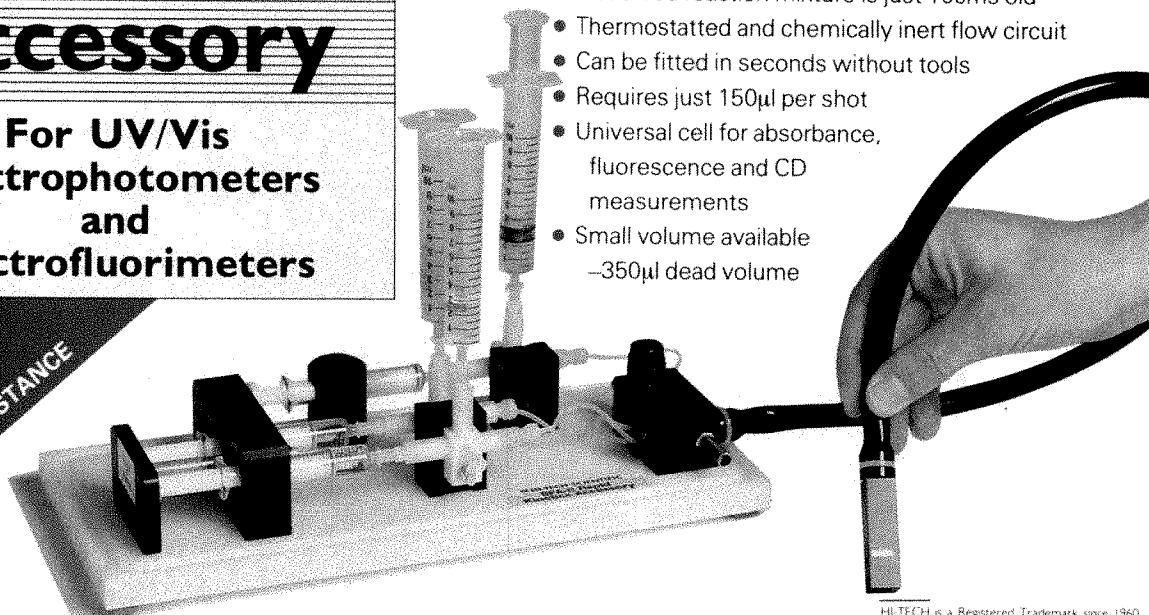
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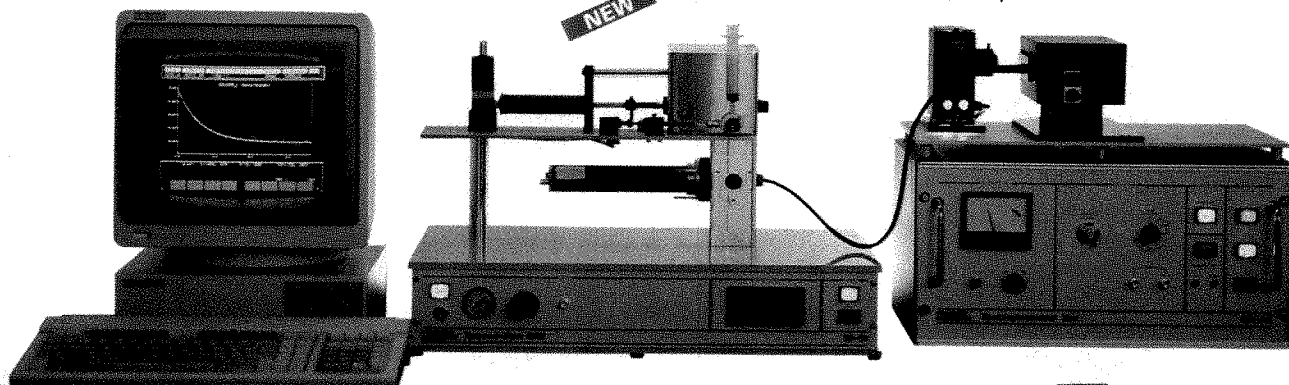
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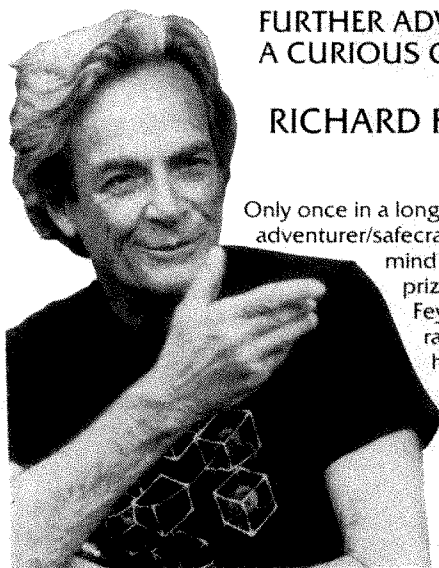
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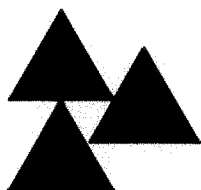
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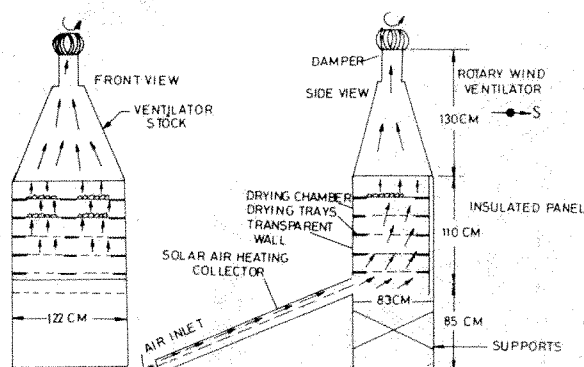


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# The rich and the irradiated

Daniel J. Kevles

**Uranium Frenzy: Boom and Bust on the Colorado Plateau.** By Raye C. Ringholz. W.W. Norton: 1989. Pp. 310. \$18.95. To be published in Britain in June, £13.95.

IN *Uranium Frenzy*, a tale of the modern American West, Raye Ringholz recounts the quicksilver dreams, leaden disappointments (and worse) brought about after the Second World War by the United States Atomic Energy Commission's desperate determination to encourage the establishment of a domestic uranium supply. The AEC, as the Commission was commonly known, offered prospectors a \$10,000 bonus for each discovery of a uranium lode and guaranteed to purchase ore production for ten years at a certain minimum price. The inducements helped to stimulate a boom in uranium prospecting, mining and production in the Colorado Plateau, the dry, unforgiving region where Colorado, Utah, Arizona and New Mexico conjoin.

The author is a longtime resident of the Plateau region and an experienced freelance author. She brings to her book a first-hand familiarity with the ambience of the boom years and information and anecdotes drawn from interviews — they form her principal sources — with many of the participants. Although attentive to the larger government policies that shaped the boom, she tells the tale by fastening on the stories of individual players, both winners (the prospectors and penny-stock jobbers who became millionaires overnight) and an array of losers. Some of the losers — the lucky ones — lost only their shirts. Others, found among the miners whose lungs were exposed to radon and its daughter products, came down, years later, with lung cancer.

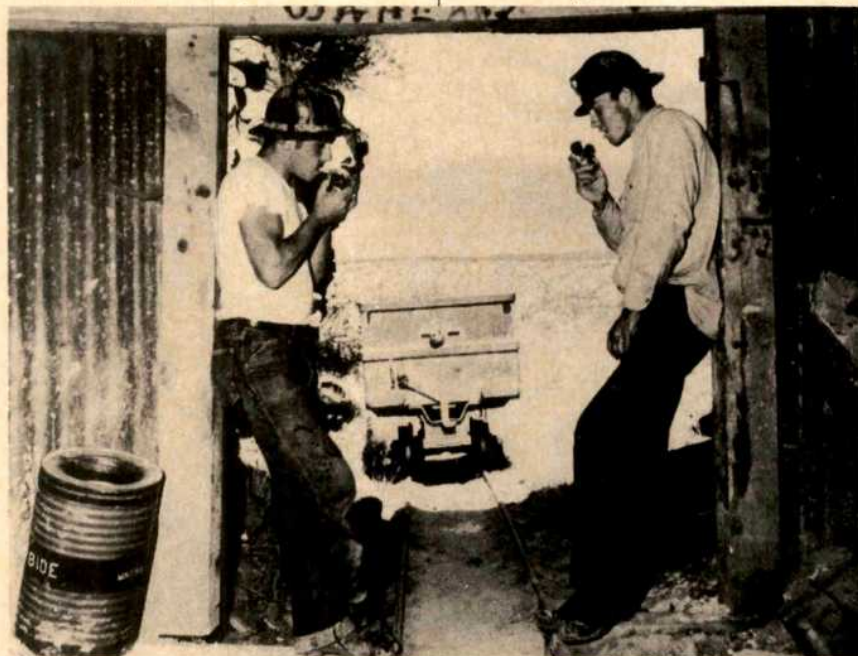
Ringholz personifies the boom with Charlie Steen, who came to the Plateau, in 1949, as a young, rebellious and unemployed geologist to hunt for uranium. He searched for the ore in areas where conventional wisdom said it didn't exist and found a rich core sample of pitchblende when, in July 1952, he broke his last drilling bit at 197 feet. Charlie and his mother, Rose, who had grubstaked him, promptly formed the Utex Corporation to exploit the strike, joining with two partners, who put \$19,000 into the venture. In December 1953, Charlie and Rose bought their partners out, paying them \$3,272,000 for the Utex stock they had received for their \$19,000 investment slightly more than a year earlier.

By this time, the uranium boom was on in earnest. Moab, Utah, where Steen and his family had moved, had been a small, conservative, inbred town; now it pulsed with the traffic of Cadillac convertibles,

ore trucks and enormous Chevrolet station wagons. In 1952, the stock-salesman population of Salt Lake City had totalled 100 people working for 20 brokerages; by March 1955, it had reached 467 salesmen hawking their wares for 80 houses. The come-ons could be, to say the least, inventive. Squeeze the pitchblende, one

mining continued. The next year, the first death from lung cancer occurred among the miners on the Plateau.

Ringholz makes clear that the federal government dealt rather less decisively with the hazards of radiation to uranium miners than it did with those of stock fraud to uranium investors. Not that federal officials were unaware of the danger to the miners: in 1950, Duncan Holaday, a member of the United States Public Health Service, had begun a study of radiation levels in the mines. By 1952, he had produced a report showing that the levels — a median in 48 mines of 3,100 picocuries of radon gas per litre of air — were far higher than those in European



Double jeopardy — uranium miners take a cigarette break.

salesman suggested, deadpan, to a prospective customer who had mentioned that she suffered from rheumatism. After 15 minutes of squeezing, she said that she felt no more pain and bought \$50,000 worth of uranium stock. On May 27, 1954, seven million uranium shares changed hands in Salt Lake City, setting a daily record. A commentator noted in the *Salt Lake Tribune*, "It's a giant slot machine with uranium shares instead of bells" (p. 109).

Investors, high on paper profits one day, were brought to earth the next by fraud and bankruptcy. Utah officials resisted regulation by the United States Securities and Exchange Commission, preferring to keep oversight of uranium stocks in local hands, which were more sympathetic than federal authorities might be to venture capital fliers. Anyway, it was said, you couldn't legislate financial morals or protection. Eventually, many Utah brokerage houses were indicted for violations of federal security regulations and a number lost their broker's licences. In 1955, the boom in uranium stocks ended, but the uranium

mines where a large percentage of workers had eventually contracted lung cancer.

No copies of the report were made available to the miners — Holaday and his colleagues had gained access to the mines by tacitly agreeing not to disseminate their findings in a way that might alarm the men down in the shafts. High officials of the AEC declined to publicize it for fear that general knowledge of the risks might jeopardize the production of uranium. Before the report was produced, and also in the years after it, Holaday urged the adoption of various safety measures — notably much-improved ventilation — to reduce the radiation hazards. Some of the larger mines responded with meliorative action, but most of the operators on the Plateau did not. All the while, the AEC insisted that it had no authority to impose safety standards. When the issue came up in 1959, the Commission typically passed the buck to state health agencies, which did virtually nothing, and to the Public Health Service, which had no regulatory leverage and whose only recourse, as Ringholz puts it, was to "implore, exhort,

From *Uranium Frenzy*.



attempt to educate" (pp. 148–150).

By the early 1960s, the miners in Holaday's study cohort were dying of lung cancer at a higher rate than expected on the basis of the incidence of the disease in the general population. In 1963, the widow of one of them, Mrs Eola Garner, started to press a legal case for compensation. In 1967, J.V. Reistrup, a reporter for the *Washington Post*, brought the plight of the miners to national attention and Willard Wirtz, the Secretary of Labor, on the authority of an old statute finally acted to set federal safety standards in the uranium mines. By 1977, when Mrs Garner at last won a favourable settlement from the state of Colorado, conditions in the mines were much safer.

*Uranium Frenzy* is not a probing analysis of high policy, but it is a fine example of high and absorbing journalistic art. It graphically depicts the behaviour and attitudes in the uranium game on the Colorado Plateau, delineating in moving counterpoint the United States govern-

ment's material success against the ill fate of the men who dug the precious ore out of the rock. Ringholz's prose is detached and clear-eyed, understated yet unsparing in its assessments of the winners, the victims, the public health officials who tried to forestall the tragedy that befell the miners — and the bureaucratic postures that prevented them from succeeding. Her book makes utterly plausible the summary of the uranium craze that Stewart Udall, the one-time Secretary of the Interior, supplied her in a letter, in November 1987: "What is most poignant is that the losers were innocent victims — and the Atomic Energy big shots were 'patriots' who lied to protect what they conceived as the 'national interest'" (p. 262). The lies may have taken the form mainly of suppressions and omissions, but they were lies, fatal lies, nonetheless. □

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## In his own time

Mark Ridley

**The Non-Darwinian Revolution: Reinterpreting a Historical Myth.** By Peter J. Bowler. *The Johns Hopkins University Press: 1988. Pp.238. \$30.25, £17.50.*

THE Reverend Thomas Malthus, who is well known for his belief that population growth must exceed the food supply, argued also that the resulting food shortage is beneficial for the human race. It raises people out of idleness and torpor. "The necessity of food for the support of life [he wrote] gives rise, probably, to a greater quantity of exertions than any other want, bodily or mental." It is these exertions that have been the motor of human progress — "Had population and food increased in the same ratio, it is probable that man might never have risen from the savage state". Malthus's ideas would develop into the familiar Victorian idea of 'progress through struggle': we desire to better our condition, we strive to do so and (provided the government follows a *laissez faire* policy) progress is the result.

Historians, and biologists, have often described Darwin's theory as an expression of the broader Victorian political economy. Strictly, this has no bearing on whether Darwin was right; but Darwin's critics have always been fond of the analogy, both for the ideological tarnish and the chance of compromising Darwin's originality. Peter Bowler's latest book is most interesting for the thorough, critical look it takes at the historical question.

Bowler has to deal with three argu-

ments. First, that Darwin's theory apparently resembles those of Malthus, and of Herbert Spencer and other Victorian writers; second, that natural selection was simultaneously discovered, at least by Alfred Russel Wallace and perhaps by Patrick Matthew and Edward Blyth, which suggests that the idea was indeed in the air; and finally, that Darwin's theory was rapidly accepted and developed into the influential politics of social Darwinism.

Bowler has objections to all three arguments. For a start, the way that the struggle for existence led to progress was crucially different in the theories of Darwin and of the political economists. For Malthus, and Spencer, the struggle worked as a stimulus to individual action, whereas in Darwin's theory selection takes place on inherited differences between individuals within a population. As Bowler neatly puts it, in Spencer's Lamarckist theory, evolution takes place in individuals, not populations; it lacks what Ernst Mayr calls 'population thinking'.

Wallace's theory may also have differed from Darwin's. In modern population genetic terms, Wallace appears to have been a group selectionist who thought of all selection as 'hard'. He was concerned with selection between "varieties", not individuals, and thought that selection only took place when a variety did not possess an adaptation needed to survive the external environment. Wallace's theory, like Spencer's, lacked Darwin's ideas of intraspecific competition and individual selection.

Finally, Darwin's theory was hardly accepted at all in the late nineteenth, or the early twentieth, centuries. What actually happened was that Darwin persuaded

biologists only about evolutionary change. They then called themselves Darwinians, but were really (what Bowler calls) "pseudo-Darwinians": they believed not in the Darwinian sort of branching, contingent, unplanned evolution, but in evolution through a series of developmental stages from protozoa to human beings. The developmental model of evolution resulted in the recapitulatory, Haeckelian kind of research in embryology, and in the theory of orthogenesis in palaeontology: both of these theories were still teleological, even if the static teleology of pre-Darwinian natural teleology had become the temporal teleology of orthogenesis. Bowler also argues that social Darwinists were inspired by Spencer's Lamarckist and progressive model of evolution, rather than by Darwin. In another chapter he describes a similar tendency in anthropology, as human evolution was considered to proceed through a series of racial stages, from savages to civilized Westerners.

Darwin thus appears as a historically isolated figure. There was no 'Darwinian revolution'. The main stream of Victorian thought started with the natural theologians and idealists, such as Richard Owen, who believed in a more or less atemporal plan of nature. It ran through Chambers, and Spencer, who tried to temporalize the plan, but proved unpersuasive. Darwin, however, was successful. He unintentionally led late-nineteenth-century biologists to accept a temporal, though still teleological, plan of nature, in which evolution proceeds through a series of stages, driven by some process of directed variation. He convinced no one either about his branching and non-progressive theory of evolution, or his materialist mechanism for it.

The 'Darwin industry' has often been criticized for concentrating on Darwin and ignoring his historical context. Bowler himself has some sharp things — not well directed, in my opinion — to say about that industry. *The Non-Darwinian Revolution* is a professional and worthwhile book, which merits reading; it offers a new (at least for the non-specialist reader) interpretation of events about which most people have opinions. I only really disagree with the way Bowler treats his fellow historians, whom he from time to time ignores or caricatures, but this is only a minor defect. Indeed, the industry itself may enjoy the last laugh. If we look past Bowler's various criticisms, and concentrate on his conclusions, he appears to have vindicated those whom he purports to oppose. Bowler has given Darwin his real Victorian context: and ends up justifying those who study Darwin in isolation. □

*Mark Ridley is in the Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.*



## Into the fishy business

J. H. S. Blaxter

**The Provident Sea.** By D. H. Cushing. Cambridge University Press: 1988. Pp. 329. £37.50, \$65.

THE history of fisheries demonstrates some of mankind's better individual qualities — the ability to innovate and organize, and ruggedness under harsh conditions — as well as some less-desirable national foibles such as aggression and greed. These human attributes are well brought out in Cushing's book, which shows that fishermen and fishery biologists have some of the better qualities in common!

The history of fishing is dealt with on a wide geographical basis and some fisheries (mainly of the Northern Hemisphere) are described case by case — for example the North Sea herring fishery, the Newfoundland cod and harp seal fisheries, the Pribilof fur seal fishery and the great fisheries for whales. The origins of fisheries research and its increasing sophistication between 1945 and 1965, and from 1965 to the present time, are examined in relation to similar phases in the development of the regulating bodies or institutions concerned.

In prehistoric times, fish were caught by hook and harpoon; nets were already in use in the Neolithic. But a well-documented expansion took place in high-seas fisheries in the Middle Ages when fish caught in the Baltic and North Sea became a major source of employment and wealth, for example for the Hanseatic towns. Soon a high degree of international organization and integration evolved, not only in the fishing itself, but in landing, on-shore processing, transport and marketing.

Certain advances can then be identified, each causing a new leap forward. The first was the invention of on-board curing of herring by the Dutch in the fourteenth century. There followed the introduction of steam tugs for towing sailing vessels in and out of port in the middle 1800s, and then steam engines for propulsion and handling gear on the fishing vessels themselves in the late 1800s. The post-1945 development of stern trawlers, freezing at sea, use of midwater trawls and purse seines, and the deployment of fishing fleets with mother ships and catchers increased the world catch enormously, leading to its probable present asymptote near 80 million tonnes a year.

A large part of the book consists of chapters on the origin of fisheries research in the last century and its progress after the Second World War. The concomitant setting-up of international regulatory bodies closely parallels the development

of research. Clearly, the initiative for research and regulation stemmed from the increasing power and efficacy of the fleets, which began to cause severe depletion of fish stocks, a problem of little importance in the pre-steam days.

As the author points out, at one time nations went to war over their fisheries; now they try to resolve their problems by appraising the scientific evidence. The story of the interplay between fishermen and fishing, and fishery scientists and regulation, is a fascinating mix of ignorance, greed, optimism, political expediency and idealism. Apart from some Scandinavian countries such as Iceland and Norway, where scientists may be held in some esteem, fishery biologists have been treated with suspicion by the fishermen, who have resented the loss of short-term profits and ignored the goal of long-term consistency of catch. The ever-increasing effect of overfishing has recently led to a greater appreciation by fishermen of the need for regulation. Earlier methods of regulation, such as minimum mesh sizes, have of late been supplemented by the imposition of quotas for vessels and total allowable catches for fisheries. Usually the medicine prescribed can easily

be agreed by the scientists on an international basis — even if the politicians find it difficult to allocate the dose to their respective countries.

Cushing does not commend his book to any particular readership. Parts of it are probably too technical for the historian or economist, or too specialized for the undergraduate. Rather, it seems ideal for MSc students on fisheries management courses, or as background reading for marine and fishery biologists as well as civil servants and politicians — throughout the world, fisheries are often now as much a political as a commercial or scientific matter. The author does not refer to the increasing importance of freshwater fisheries and aquaculture (together yielding about 11 million tonnes in 1986), nor does he attempt to forecast the future. Although such additions would have enhanced the value of the book, it already contains an unusual and useful mixture of history, economics and science. It will no doubt find its way into many libraries — not least, I hope, those of certain government departments. □

J. H. S. Blaxter is Deputy Chief Scientific Officer at the Dunstaffnage Marine Laboratory, PO Box 3, Oban, Argyll PA34 4AD, UK.

## The Great Dane

H.B.G. Casimir

**Harmony and Unity: The Life of Niels Bohr.** By Niels Blaedel. Science Tech Publishers, 701 Ridge Street, Madison, Wisconsin: 1988. Pp. 323. \$35. Distributed outside North America by Springer-Verlag, DM98, £32.

NIELS BOHR was one of the greatest physicists of our century. His papers on the spectrum of hydrogen, in which he postulated the existence of stationary states and of quantum jumps, inaugurated the era of the quantum theory of line spectra, led to an understanding of the periodic system of the elements — another notable contribution of Bohr's — and finally to the formulation of quantum mechanics. Bohr did not contribute to the mathematical formalism of the new mechanics, but his doctrine of 'complementarity' played an essential role in clarifying its interpretation. He then turned from atomic to nuclear physics, introducing the notion of highly excited intermediate states in nuclear reactions and explaining many features of uranium fission. Through his writings, and even more through personal discussions, he had an enormous influence on younger physicists.

During the Second World War Bohr escaped from his native Denmark, reaching the United States by way of Sweden and England. He was keenly aware of the



Niels Bohr — an enormous influence on younger physicists.

decisive role nuclear weapons were going to play in international politics, and foresaw with great clarity the threat of a cold war, but his attempts to convince Roosevelt and Churchill that they should come to terms with the Soviet Union at an early date were unsuccessful.

He was certainly a true citizen of the world. But, as one of his biographers, quoted by Blaedel, remarks, he was so deeply rooted in Danish culture and the Danish way of thinking and feeling that, if he had grown up in some other country, he would not have been Niels Bohr. He was also the head of a wonderful family. ▶



There is no lack of published material about Bohr. The edition of his collected papers also contains many biographical details, letters and so on, and there have been numerous obituaries and at least two commemorative volumes. In this book, a translation and slight revision of the Danish edition of 1985, Blaedel has addressed himself to the task of writing a full-length biography that covers all facets of his subject and that emphasizes that they form part of one harmonious unity. I think that on the whole he has succeeded remarkably well. He gives an accurate picture of the man theorists of my generation both admired and loved. And not only of the physicist: Bohr's relations with his family and in particular with his wife, an admirable woman, are drawn with sympathy and understanding.

Blaedel's sketch of the atmosphere at Bohr's institute in Copenhagen, where work was combined with — often somewhat childish — playfulness, especially in the late 1920s and early 1930s, is true to life; it will raise nostalgic memories among those who, like myself, experienced it. I am not so sure about the physics, however. The essential subjects are included and I have not spotted obvious errors, but I wonder whether Blaedel's account of the discussions between Bohr and Einstein — to give one example — will be comprehensible to non-physicists. Here, though, is a book to be read as a biography and not as an introduction to modern physics.

The translation from the Danish is in general satisfactory, although I noticed one or two slips. A few errors in the original have been corrected, and here and there short explanations have been added to help readers who are unfamiliar with Danish geography and Danish history and literature. The book is richly illustrated; although the quality of reproduction is not as high as that of the rather sumptuous original (which also contains a few colour plates), it is perfectly adequate.

Some readers may regret that the book is more a eulogy than a critical appraisal — that Blaedel does not mention any scientific shortcomings or any less desirable human traits. So be it. Personally I think he has produced a fitting tribute to a great scientist and a noble man. □

*H.B.G. Casimir, De Zegge 7, 5591 TT Heeze, The Netherlands, studied theoretical physics at Leiden, Copenhagen and Zürich, and from 1946 to 1972 was Research Director of the Philips Company at Eindhoven.*

#### New in Britain

Just published by Unwin Hyman, price £11.95, is *"What Do You Care What Other People Think"* by Richard Feynman as told to Ralph Leighton. The book, which was published last autumn in the United States by W.W. Norton and reviewed in *Nature* on 3 November, tells largely of Feynman's productively unconventional work on the inquiry into the failure of the Challenger space shuttle.

## Wholesome ideals

*P. W. Atkins*

**Science, Order and Creativity.** By David Bohm and F. David Peat. *Routledge: 1988. Pp.280. Pbk £5.95.*

THE world will be much nicer once 'creativity' has been liberated from its present tomb, the walls of which are built from the attitudes of conventional science. This is the message that David Bohm and F. David Peat urge on us in this somewhat bizarre and certainly uneven book that has grown out of conversations that the two have had together over the years. I began to read with high hopes. But although some of the early passages show interesting insights, in general my dislike grew with the page numbers.

The book is pervaded on the one hand by a schoolboy naïveté ("How does science intend to end the danger of mutual annihilation that exists in the world?") and on the other by a pessimism about the potential of and the attitudes currently struck by conventional science. Before considering purchasing it, those who are sensitive to these things will want to know that both Sir Fred Hoyle and Rupert Sheldrake are mentioned without disapproval.

To be fair, I must allow the authors to speak for themselves, for I fear I may be one of those at whom is directed the accusation of being a prisoner of conventional science, and therefore may be over-tender to its message. The argument begins with an attempt to disarm criticism of what is to come by considering the role of communication in creative perception, and by identifying how communication may break down at the dawn of revolutions in science and at what the authors so fervently long for, the sunrise of a new paradigm. Quantum theory is in this context a paradigm of paradigms, and something of the wars of interpretation is presented here. Rather inappropriately, though, Bohm takes the opportunity to present a moderately lengthy account of his 'causal interpretation', for which he needs the burden of a new potential (the quantum potential, essentially  $\nabla^2\psi^2/\psi^2$ ). The advantage of his formalism appears to be that it does not require a break with classically established concepts; here, however, the authors have tripped over their own feet, for a more relaxed view of quantum theory would lead us to believe that we must unburden ourselves of the expectation of the farmyard and not cling to classical concepts.

Built on these foundations, such as they are, is an account of what is meant by order, its classification and (an essential feature of what is to come) its unfolding.

The authors deal properly with the change in our perception of the concept that has been stimulated by developments in fractals and determinate chaos. To do so, they distinguish between 'constitutive order' (the actual order expressed in the physical constitution of the entity, as in the material hexagons of a beehive) and 'descriptive order' (the order that stems from an abstraction, as in the motion of an object through a field of coordinates). They argue that actual order (whatever that is) lies between these two extremes, and thus alight on what is for them the comforting view that order is a cycle of activity that involves both. It is in this chapter that the generally 'Eastern' flavour of the book begins to be explicit for the first time.

The authors are desperate to eliminate the fragmentation of conventional science and thus to impress upon us the essential 'wholeness' that is crucial to comprehension and human progress. Hence order, of various degrees, kinds and hierarchies, is essential to their argument. Thus they go on to distinguish 'generative order' from 'implicate order'. The former is an "inward order out of which the manifest form of things can emerge creatively". The rule for generating fractals would be an example. The latter is a particular kind of generative order that, I must confess, leaves me confused, for to comprehend it we must somehow understand that "the basic movement of enfoldment and unfoldment is thus a dual one in which there is ultimately no separation between enfoldment and the unfoldment". There is, of course, a superimplicate order too.

In the tacit infrastructure of consciousness, the authors claim, there are rigid ideas that restrict creativity. (I suspect, on the contrary, that they constitute a ladder to comprehension.) These ideas operate close to the source of the generative order and, as well as restricting creativity, imply "the presence of an energy that is directed toward general destructiveness". Whatever that means, I feel that it is the manifestation of the authors' pessimism, and more truly the spur of their writings than the overt joy of enlarged comprehension that they claim to be the book's impetus.

Bohm and Peat urge on us the "key importance of liberating creativity" if human life is to have a worthwhile kind of survival. And how do we escape from our prison? We need to de-fragment science, attain wholeness, comprehend levels of order, give up the hope of true and total comprehension, and, perhaps most important of all, "clear up" the sociocultural dimension. It really is as easy as that. □

*P.W. Atkins is in the Physical Chemistry Laboratory, University of Oxford, and is a Fellow of Lincoln College, Oxford OX1 3DR, UK.*

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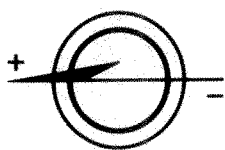
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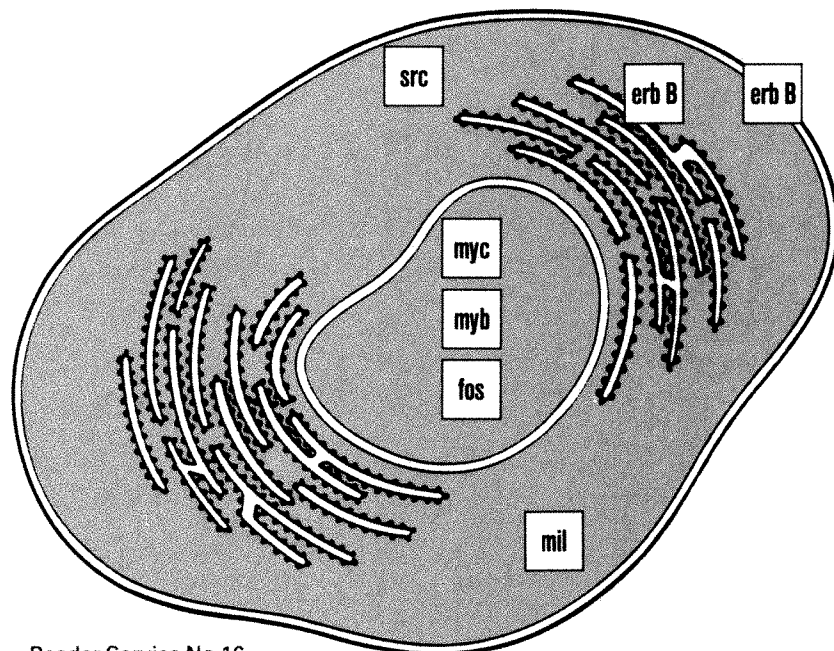
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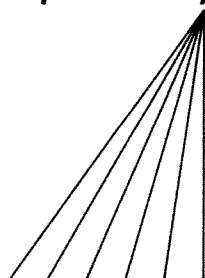
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# Geochemical implications of the formation of the Moon by a single giant impact

Horton E. Newsom & Stuart Ross Taylor

The origin of the Moon by a single massive impact of a body slightly larger than Mars with the Earth can explain the angular momentum, orbital characteristics and unique nature of the Earth–Moon system. The density and chemical differences between the Earth and the Moon are accounted for by deriving the Moon from the mantle of the impactor. A cosmochemically plausible impactor can be formed in the region of the inner Solar System, lending support to the impact hypothesis.

THE Earth–Moon system has several unique features. None of the other terrestrial planets possesses a comparable moon—the tiny martian moons, Phobos and Deimos, are probably captured asteroids. The angular momentum of the Earth–Moon pair is anomalously high compared with that of the other inner planets, and the inclination of the lunar orbit is strange. The Moon has a high mass (1/81.3) relative to the Earth when compared with the satellites of the giant planets, but its bulk density ( $3.34 \text{ g cm}^{-3}$ ) is much lower than that of the Earth or the other inner planets, probably because of a low metallic iron content.

The chemical composition of the Moon, revealed by the samples from the Apollo and Luna missions, is also unusual. The Moon is bone dry, is strongly depleted in volatile elements such as potassium, lead and bismuth, and probably enriched in refractory elements such as calcium, aluminium, titanium and uranium (see box). In bulk, it contains ~50% more FeO than the upper mantle of the Earth.

In view of this diverse set of properties, it is perhaps not surprising that theories for the origin of the Moon have generally been unsatisfactory. Only recently has a model been proposed that is widely considered to be acceptable: the single impact hypothesis. This theory proposes that during the final stages of the accretion of the terrestrial planets, a body somewhat larger than Mars collided with the Earth and spun out a disk of material from which the Moon formed. Here we will review the evidence for this theory and show how it can account for the unusual chemical composition of the Moon.

## Older hypotheses

Including the single impact theory, there are five main classes of hypothesis for the origin of the Moon, although elements of some appear in others. The four older hypotheses fail to explain the unique nature of the Moon because they do not account for the lunar orbit or the high angular momentum of the Earth–Moon system, and because they entail processes that might have been common in the early Solar System, implying that similar satellites should accompany all the inner planets.

Capture of an already formed Moon from an independent orbit is improbable on dynamical grounds<sup>1</sup> and does not explain

the compositional peculiarities, as the Moon would be expected to be an example of a common early Solar System object<sup>2</sup>.

Formation of the Earth–Moon system as a double planet immediately stumbles on the difficulties of density and composition. To overcome the density problem, co-accretion was proposed<sup>3</sup>; in this model the Moon formed from a ring of low-density silicate debris shed from the mantles of incoming differentiated planetesimals, whose iron cores survived to accrete with the Earth. Although attractive, this scenario is improbable<sup>1</sup> because the breakup of planetesimals is unlikely to occur and it is difficult to achieve the required angular momentum<sup>4</sup>. In a similar model for co-accretion and evolution of a circum-terrestrial disk<sup>5,6</sup>, it has been shown that the angular momentum is attained only in very special conditions.

Fission hypotheses, which derive the Moon from the terrestrial mantle<sup>7</sup>, have been popular because they explain a low-density, metal-poor Moon, but they had to be abandoned after lunar samples showed significant differences in chemical composition between the Moon and the Earth's mantle<sup>8</sup>. A modification of this hypothesis<sup>9,10</sup> suggests that mantle material was thrown into orbit by many small impacts. Apart from the inherent chemical difficulties, it is exceedingly difficult to obtain the required angular momentum this way<sup>1,11</sup>. A further elaboration of this proposal, involving capture of objects in heliocentric orbits by an extended Earth atmosphere<sup>9</sup>, is not supported by existing evidence<sup>8,12,13</sup>, and this model also fails to account for the unique status of the Moon.

## Single impact hypothesis

Formation of the Moon as the result of a single collision of a large body with the early Earth resolves many of these problems. The theory was first proposed to account for the anomalous angular momentum<sup>14</sup>, but also provides an explanation for the other properties and has now become widely accepted<sup>15</sup>.

In assessing the single impact theory, one must first ask whether a suitably large body existed in the early Solar System (before 4.4 Gyr). One scheme for the origin of the terrestrial planets<sup>12</sup>, in which they are accreted from a suite of rocky planetesimals over a period of  $10^7$ – $10^8$  yr, leads to a hierarchy of large bodies. In the final stages of accretion, perhaps 100 objects of lunar mass, 10 of the mass of Mercury and a few Mars-sized bodies populate the inner Solar System<sup>12</sup> before being swept up into the four inner planets.

There is considerable evidence for this scheme. Craters and ringed basins over 1,000 km in diameter on the Moon, Mercury, Mars and the satellites of the outer planets<sup>16</sup> attest to an early (>3.8 Gyr) intense bombardment by a large range of objects. The axes of nearly all the planets are significantly tilted relative to the plane of the ecliptic; the most dramatic example is Uranus, which is lying on its side, probably as a result of a collision with an Earth-sized object. The slow backward rotation of Venus, unique in the Solar System, is most rationally attributed to a

### Classification of the chemical elements

To describe the behaviour of the elements in the solar nebula, cosmochemists divide them according to their volatility (examples in parentheses): 'gaseous' (hydrogen, carbon, nitrogen, oxygen and the noble gases), 'very volatile' (bismuth, thallium), 'volatile' (rubidium, caesium), 'moderately volatile' (potassium, manganese), 'moderately refractory' (vanadium, europium), 'refractory' (calcium, aluminium, uranium, lanthanum) and 'super-refractory' (zirconium, scandium). The terms lithophile, chalcophile and siderophile describe those elements that preferentially enter silicate, sulphide or metal phases, respectively. This classification is based primarily on the distribution of elements in these phases in meteorites.



late collision with a massive, perhaps Mars-sized object; with a different mass, angle and velocity, the impact might have provided Venus with its own moon.

Further evidence comes from the varied compositions of the planets; accretion from a dusty nebula, rather than from planetesimals, might be expected to produce rather uniform planetary compositions. Meteorites, too, come from many distinct parent bodies and are commonly mixtures of several components, or fragments of larger bodies. The asteroid belt, arrested at an early stage of planetary development, probably owing to the gravitational influence of the gaseous giant Jupiter, preserves a wide size range of objects (the largest, 1 Ceres, is 1,020 km in diameter). Finally, Mercury's anomalously high density and large iron core are most simply explained by removal of most of its silicate mantle by collision with a large body<sup>17</sup>; alternative schemes involving high-temperature evaporation of silicates remove unrealistic amounts of material<sup>18</sup> and do not account for the presence of sodium ions, probably sputtered from the surface, in Mercury's tenuous atmosphere<sup>19</sup>.

### Impact dynamics

Studies of the single impact hypothesis<sup>20-26</sup> have led to the following sequence of events (see Fig. 1). When the Earth had attained nearly its present size, it suffered a grazing impact, at about  $5 \text{ km s}^{-1}$ , with an object of  $\sim 0.14$  Earth masses, somewhat larger than Mars<sup>20,21</sup>. Both this body and the Earth are assumed to have differentiated into a metallic core and silicate mantle. The collision disrupts the impactor, much of which goes into orbit around the Earth, accelerated by the gravitational torques arising from the asymmetrical shape of the Earth following the impact<sup>20,21</sup>, and by expanding gases from the vaporized part of the impactor<sup>14,23</sup>. While the impactor's mantle is being accelerated away from the Earth, its metallic core separates from the mantle, decelerates, and accretes to the Earth in about four hours<sup>22</sup>.

The material that achieves orbit is initially present as a disk partly inside and partly outside the Roche limit ( $\sim 2.9$  Earth radii)<sup>22,25,26</sup>. Some of the material inside the Roche limit ends up outside through transfer of angular momentum. The material may immediately coalesce to form a totally molten Moon, or break up into several moonlets which then accrete to form a partly molten Moon<sup>26</sup>. Geochemical studies indicate that at least half the Moon was molten shortly after accretion, with the feldspathic highland crust crystallizing from this 'magma ocean'<sup>27</sup>; in general, a partly rather than fully molten Moon seems most consistent with geochemical and geophysical constraints<sup>26</sup>.

### Lunar composition

Only a small amount of material from the Earth's mantle (probably less than 16%<sup>20,22</sup>) eventually ends up in the Moon. This is supported<sup>8</sup> by the low FeO content of the terrestrial mantle (8%) as compared with the Moon (13%), which would otherwise require that the FeO content of the impactor mantle be unreasonably large ( $>13\%$ )<sup>28</sup>. A Moon formed from 85% impactor mantle and 15% terrestrial mantle requires an impactor mantle with 14% FeO.

Additional evidence that there is relatively little terrestrial mantle material in the Moon comes from the isotopic composition of potassium<sup>29</sup>. Because the Moon is depleted in volatile elements relative to the Earth's mantle, the mass fractionation due to volatile loss should have left the Moon enriched in the heavier potassium isotopes. The fact that the isotopic composition of potassium in lunar feldspars is close to that of CI carbonaceous chondrites, considered to be typical of the solar nebula<sup>29</sup>, limits the contribution from the Earth's mantle to  $<20\%$ .

Relative to chondritic abundances (see Fig. 2 legend for a discussion of the use of carbonaceous chondrites as 'benchmarks'), the Moon and the Earth's mantle show similar depletion

patterns of vanadium, chromium and manganese<sup>9,30</sup>. These contrast with the more chondrite-like abundances inferred for the parent bodies of the eucrite and shergottite meteorites, and are considered by some<sup>9,10</sup> to support an origin of the Moon from the Earth's mantle. But the depletion of Mn relative to V and Cr in the Earth and the Moon is also consistent with volatility having been the primary control on the abundance pattern<sup>31,32</sup>. New experiments on partitioning of these elements between sulphur-rich metallic liquid and silicate melt<sup>31,32</sup>, confirming earlier work on partitioning between pure iron metal and silicate melt<sup>33,34</sup>, as well as data for Cr and Mn in natural metal-silicate rocks from Disko Island<sup>35</sup>, show that V and Cr are more highly siderophile (literally, 'iron-loving'; see box) than Mn. Thus, had these elements been depleted by core formation in the Earth, we would see a greater depletion of V and Cr compared to Mn, contrary to observation. Their depletion in the Earth's mantle, therefore, cannot be ascribed to unique terrestrial processes involving formation of the Earth's core<sup>9,36</sup>, and similar depletions of V, Cr and Mn in the Earth and Moon do not require that the Moon formed primarily from terrestrial mantle material.

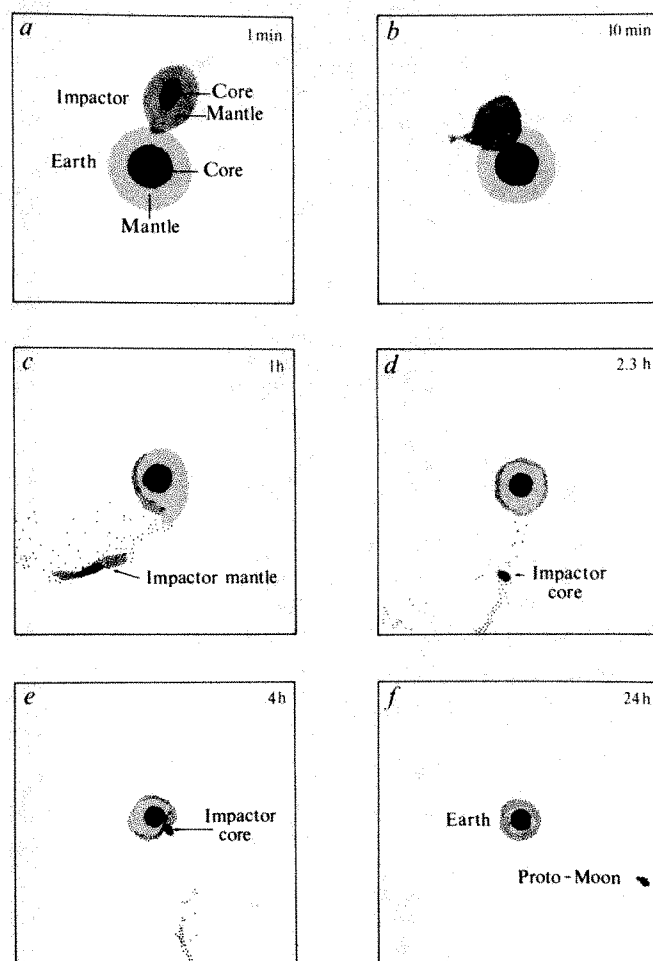


FIG. 1 Computer simulation<sup>20-22</sup> of the formation of the Moon by a giant impact. This reconstruction shows the events following the oblique collision with the Earth of an object of 0.14 Earth masses (slightly larger than Mars) at a velocity of  $5 \text{ km s}^{-1}$ . Both the Earth and the impactor have already differentiated into a metallic core and silicate mantle. The time elapsed since impact is given in each box. Following the collision (a, b), the impactor spreads out in space (c), but the debris clumps together through gravitational attraction. The iron core of the impactor separates from the silicate mantle (d) and accretes to the Earth (e) about four hours after the initial encounter. Nearly 24 hours later (f), a silicate lump of about lunar mass is in orbit around the Earth. This material is principally derived from the silicate mantle of the impactor. Courtesy of A. G. W. Cameron and W. Benz.

## Constraints on impactor composition

What can be deduced about the composition of the impacting body? We will first consider the constraints on the lithophile and volatile element composition of the impactor, and then use the siderophile element depletion patterns in the Earth and Moon to derive the siderophile abundances in the impactor's mantle and the size and composition of the impactor's core. We assume<sup>20-22</sup> that the impactor was 0.14 Earth masses, and was differentiated into a metallic core and silicate mantle. We also assume that it formed in the same part of the solar nebula as the Earth (between Venus and Mars), to account for the low relative impact velocity ( $5 \text{ km s}^{-1}$ ; ref. 23) and the similarity in oxygen isotopes between Earth and Moon. Although there was probably much mixing of planetesimals during their formation, the terrestrial planets retain some memory of having accreted

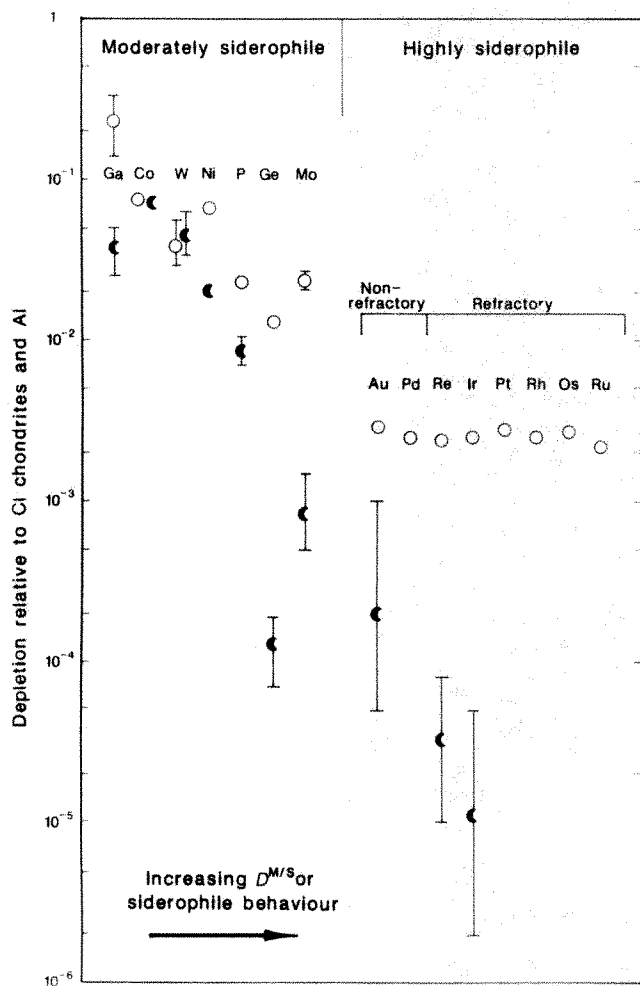


FIG. 2 Depletion of siderophile elements in the Earth (○) and Moon (●)<sup>42,51</sup>, plotted in order of metal/silicate partition coefficient,  $D^{M/S}$ . The dividing line between moderately and highly siderophile behaviour is taken to be  $D^{M/S} = 10,000$ , meaning that the element is partitioned into the metallic phase with a concentration ratio (metal:silicate) of 10,000:1. The depletions are normalized to abundances in CI chondrites relative to aluminium, because the non-siderophile refractory elements are enriched in the Earth and Moon. The greater depletion of gallium and germanium in the Moon relative to elements with similar siderophile behaviour is probably due to their volatility. Elemental abundances in Solar System objects are usually expressed relative to those in the primitive, volatile-rich meteorites known as CI chondrites. For the non-gaseous elements, these meteorites give the best estimate of the composition of the Sun, and thus of the primitive solar nebula from which the planets formed<sup>56</sup>. Primitive material, unchanged since the formation of the solar nebula, would have flat or 'chondritic' patterns; conversely, any deviation from chondritic abundances (or relative abundances) provides information about an object's chemical history.

from relatively narrow ( $<1 \text{ AU}$ ) feeding zones, a notion supported by the zoned compositional structure of the asteroid belt<sup>37</sup>. **Lithophile and volatile elements.** The constraints imposed by these elements on lunar origins and the single impact hypothesis are only briefly summarized here; for more details, see ref. 8. The impact was sufficiently energetic to vaporize much of the material that went to make up the Moon, explaining such unique features as the bone-dry nature of the Moon and the extreme depletion of very volatile elements. Bismuth and thallium, for example, are depleted by factors of  $\sim 200$  relative to cosmic abundances. Elements that are volatile at temperatures above  $\sim 1,100 \text{ K}$  (such as europium and ytterbium) do not appear to be depleted in the Moon<sup>8</sup>, setting an upper limit on the temperature experienced by proto-lunar material.

If the impactor formed in the region of the terrestrial planets, then it must have been somewhat depleted in volatile elements, as there was an earlier, if less dramatic, depletion of these elements in the inner solar nebula. This is shown by the low volatile/refractory element ratios (for example,  $\text{K/U} = (1-2) \times 10^4$ ) in the Earth, Venus and Mars, compared with initial solar nebula values of  $6 \times 10^4$ .

This depletion event must have occurred very soon after the formation of the Solar System ( $T_0 = 4.56 \text{ Gyr}$ ), as shown by the low initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in meteorites<sup>38,39</sup>, which indicate a very early separation of volatile rubidium from refractory strontium. Also, the lunar initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio is close to that of the basaltic achondrites—meteorites which formed as igneous rocks 4.54 Gyr ago<sup>38,39</sup>; thus, lunar  $^{87}\text{Sr}$  must have evolved in a low-Rb/Sr environment from times close to  $T_0$ .

Accordingly, the Rb/Sr ratio was probably lower than chondritic in the lunar precursor material. As the Earth, the Moon and the CO, CM and CV carbonaceous chondrites display similar V-Cr-Mn patterns (see above), this widespread volatility-related depletion may have been caused by early volatile depletion in the nebula, rather than the giant impact. It seems safe to conclude that the impactor mantle was not composed of material with the composition of CI chondrites, but had undergone a volatile depletion similar to that seen elsewhere in the inner Solar System<sup>8,37</sup>, and thus had Rb/Sr ratios, V-Cr-Mn patterns and K/U ratios similar to those of the inner planets; additional potassium, other volatile elements and water were lost in the collision. The physics of volatile depletion during lunar formation needs further study<sup>25</sup>.

The bulk Moon is probably enriched in refractory elements (such as Al, Ti, Ca and U) by a factor of 1.5 compared with the terrestrial mantle, or about 2.5 times the primitive abundances in CI meteorites. The geochemical arguments for this<sup>8</sup> have been buttressed by geophysical studies, which support a high-alumina Moon<sup>40</sup>. The most recent refinement of the geophysical data<sup>41</sup> concludes that "only in the case of extreme assumptions can critical aspects of bulk lunar composition be demonstrated to be equivalent to the present-day terrestrial mantle: specifically the Moon has an  $\text{Mg}\# [= \text{Mg}/(\text{Mg} + \text{Fe})]$  that is too low and an alumina abundance that is too high".

Whether the mantle of the impactor was enriched in refractory elements is less certain; alternatively, fractional condensation from a vapour phase could have enriched the Moon in alumina, uranium and the other refractory elements<sup>8</sup>. But such a process might alter isotopic ratios, and no such effects have been observed in the potassium isotopes<sup>29</sup>. We conclude that the impactor was probably enriched in refractory elements, noting that the Earth is enriched over CI abundances by a factor of 1.5. This raises broad questions about the relative compositions of the terrestrial planets, selective accretion from already differentiated planetesimals, and the effects of massive collisions that remain for future research.

**Siderophile elements.** The depletion patterns of siderophile elements in the Earth and Moon (Fig. 2) provide constraints on their abundance in the mantle of the impactor and on the size of the impactor's core. Two important characteristics of the



siderophile abundances in the Earth's upper mantle are that the highly siderophile elements (such as rhenium and iridium) seem to be uniformly distributed throughout the upper mantle, and have chondritic relative abundances (Fig. 2). These facts are often attributed to accretion of a late (post-core-formation) veneer of meteoritic material to the terrestrial upper mantle<sup>45</sup>, because the siderophile element concentrations are too high to have been in equilibrium with a metallic core.

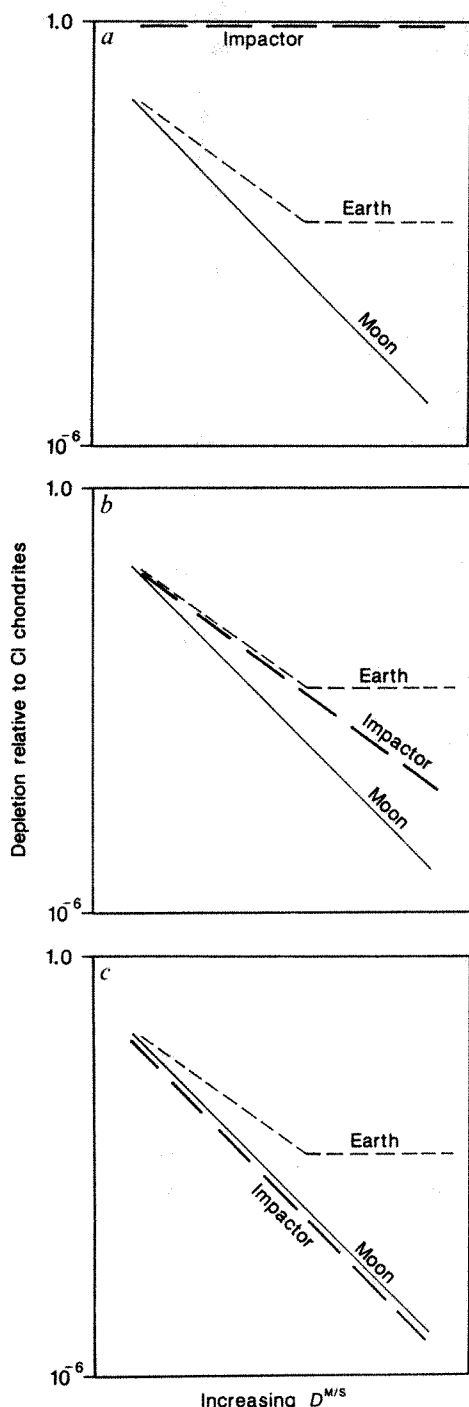


FIG. 3 Schematic siderophile element depletion patterns for the Earth and Moon, resulting from different siderophile depletion patterns in the impactor mantle. *a*, An impactor resembling a chondritic meteorite, with no segregation into metallic core and silicate mantle. *b*, An impactor mantle with a siderophile element pattern similar to that of the Earth's mantle, without the 'late veneer' of highly siderophile elements. *c*, An impactor with a siderophile element depletion pattern similar to that of the lunar mantle.

The lunar siderophile pattern, by contrast, shows a relatively uniform decrease in abundance (relative to chondrites) of the refractory siderophile elements as a function of their siderophile nature. (Elements such as gallium and phosphorus may also be depleted owing to their volatility.) This depletion pattern is quantitatively consistent with equilibrium between the lunar silicates and Fe-rich metal at low degrees of partial melting<sup>42</sup>, presumably during core formation. (The existence of a lunar core of 350–500 km radius, although not conclusively proven, is consistent with many geophysical data, including the Moon's moment of inertia coefficient<sup>43</sup>, electrical conductivity, and the latest assessment of lunar palaeomagnetism, which seems to require a dynamo in an electrically conducting core between ~4 and 3.5 Gyr ago<sup>44</sup>.) Only the least siderophile elements, such as tungsten and cobalt, are similarly depleted in the Earth and Moon.

The conclusion that core formation can explain the lunar siderophile abundances depends on the lunar core having a relatively low nickel content (<10 wt%<sup>42</sup>). A nickel-rich core would be less efficient at scavenging siderophiles from the silicate phase; thus, if the lunar core were in fact to contain 41 wt% Ni, as has recently been suggested<sup>46</sup>, the Moon's siderophile depletion would have to be explained by its formation largely out of material from the Earth's (already depleted) mantle. We do not feel that this conclusion is required, however, because the figure of 41% was derived from the Ni abundance in Apollo 15 green glass, assuming<sup>46</sup> that this glass represents a liquid from the primitive lunar mantle. In fact, the chemistry of the glass—notably its depletion in europium and strontium, characteristic of other lunar mare basalt samples<sup>27</sup>—indicates that its source region was formed by crystallization from the lunar magma ocean. If this magma ocean was in direct equilibrium with the lunar core, then the Ni content of the green glass would indeed reflect that of the lunar core. This is unlikely, however, because the metal separation would have occurred at some moderate degree of partial melting, whereas the magma ocean probably represents a high degree of melting of the lunar mantle. Assuming that a large proportion of the Moon (or precursor planet) experienced metal segregation at 10% partial melting, following which the silicates were partially or completely melted to form a magma ocean, the nickel content of the metal in the lunar core could range from 10 to 27 wt%.

An additional argument against Ni-rich metal in equilibrium with lunar silicates comes from the very reduced oxidation state of the Moon, which is characterized by a total lack of ferric iron. Lunar basalts crystallized at oxygen fugacities four to five orders of magnitude lower than those of terrestrial basalts at equivalent temperatures<sup>47</sup>. If lunar silicates had equilibrated with Ni-rich metal, lunar oxygen fugacities would be much higher.

The lunar siderophile element pattern allows four possibilities for the siderophile abundances in the impactor, the implications of which are discussed next. The second and third cases seem most plausible.

**An undifferentiated impactor.** The least likely possibility is that the impactor had chondritic abundances of siderophile elements in its mantle; that is, there was no separation of metal from silicate (Fig. 3a). Not only would the lunar iron content derived from such a source be too high, but it is hard to see how a planet larger than Mars could escape differentiation into a separate core and mantle<sup>48</sup>. There is abundant evidence from over 60 varieties of iron meteorite that core formation occurs even in relatively small asteroidal bodies. Also, the absence of a core in the impactor would make it more difficult to place a lunar mass of material in orbit<sup>20–22</sup>. The objections to an undifferentiated chondritic impactor, and to an oxidized chondritic impactor containing no Fe–Ni metal, are discussed in more detail in ref. 49.

In this model the entire siderophile element depletion observed in lunar silicates has to be explained by core formation

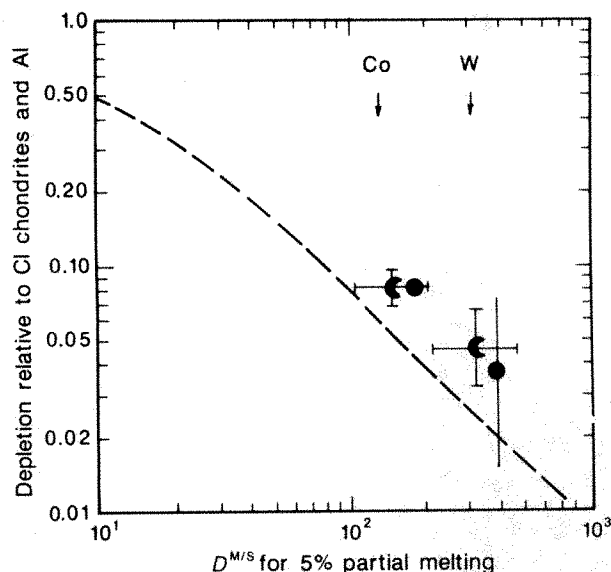


FIG. 4 The maximum core size in the impactor is constrained by the similar abundances of W and Co in the Earth (●) and Moon (○). These imply that the impactor also had similar concentrations of W and Co. The maximum allowable core size (10 wt%) corresponds to the minimum metal/silicate partition coefficients ( $D^{M/S}$ ) for these elements, and the maximum depletion<sup>42</sup>. The minimum partition coefficients are calculated for a composition of 50 wt% S-rich metal and 50 wt% Fe-metal<sup>42,54</sup>; a larger amount of S-rich metal would reduce the partition coefficient of W too much relative to Co. The maximum depletion for these elements is derived from estimates of the uncertainties of the depletions<sup>42</sup>. The dashed line represents the depletions expected for a core size of 10 wt%, and this line just grazes the maximum depletions observed in the Earth and Moon.

in the Moon. Newsom<sup>42</sup> concluded that the observed lunar depletion could be explained by segregation of ~4–5.5 wt% Fe–Ni metal<sup>40,41,50</sup>, assuming segregation at low degrees of partial melting of the silicates (5–9%).

**Siderophile depletion in impactor mantle similar to Earth's mantle.** With the exception of the highly siderophile elements (Fig. 3b), this could be produced by segregation of a small (<10 wt%) core, assuming equilibrium between the impactor's core and mantle. In the absence of core–mantle equilibrium, as in the Earth, any size of core would be possible, including 30 wt%<sup>20–22</sup>. If the Moon formed from material with a siderophile element pattern characteristic of the Earth's mantle, including Earth-like abundances of highly siderophile elements, then segregation of additional metal (<0.2 wt%) is needed in the Moon to produce the observed lunar pattern<sup>42</sup>.

**Siderophile depletion in impactor mantle similar to present Moon.** Formation of the Moon from material with essentially its present abundance of siderophile elements constrains the amount of metal in the impactor that could produce the depletion. Assuming impactor core–mantle equilibrium and the largest possible depletion of Co and W in lunar silicates, the maximum size of the impactor core is ~10 wt% metal (Fig. 4). The composition of the impactor core is also constrained by the Co and W abundances. The metal/silicate partition coefficients<sup>51</sup> are lower for sulphur-rich metal (25–30% S), allowing larger core sizes than for sulphur-free metal (Fig. 3c). Assuming that the impactor core consists of both S-rich and S-poor metal, the fraction of S-rich metal in the core must be <50%. A larger amount of S-rich metal would reduce the partition coefficient for W too much relative to Co, and W would not have been depleted, as is observed for the shergottite parent body<sup>52,53</sup>. In this model, no metal segregation in the Moon is required to explain the siderophile abundances. If a lunar core exists, as indicated by the geophysical data, there must have been disequilibrium

between the core and the lunar mantle during core formation. *Siderophile depletion in impactor mantle greater than in the Moon.* This possibility is unlikely, as it requires siderophile elements to be added to the Moon from elsewhere. The moderately siderophile elements Co and W have similar abundances in the Earth's mantle and the silicate portion of the Moon (Fig. 4). If the impactor had W and Co abundances significantly below the lunar (and terrestrial) level, addition of terrestrial material would not bring the abundance up to the lunar level. The abundances of the highly siderophile elements could be consistent with the addition of a relatively large amount of terrestrial mantle material to the Moon (15–50%), although additional complications arise if the Earth's mantle already possessed its veneer of highly siderophile elements.

### Implications for the Earth

The implications for the Earth of the single impact origin of the Moon are considerable. The event probably triggered or enhanced complete melting of the Earth's mantle<sup>26</sup>. In addition to the accretion of the impactor's core, the impactor's mantle would have provided about 10% of the mass of the Earth's mantle.

For the second and third models above, with terrestrial or lunar siderophile abundances in the impactor, the implications for the siderophile element budget of the Earth depend on the fate of the metal core from the impactor. Models of the collision<sup>20–22</sup> indicate that most of the impactor core ends up in the Earth, with the metal penetrating the mantle and wrapping around the Earth's core. This would not disturb the siderophile abundance patterns already present in the Earth's mantle, but a significant amount of material from the impactor's core, enriched in siderophile elements, would probably have been vaporized and redistributed into the mantle. The detailed implications for terrestrial siderophile abundances depend on the fraction of the accreting metal core with small enough grain size to equilibrate with the mantle, but partial retention of metal from the impactor could explain the entire siderophile element pattern in the Earth's mantle<sup>49,54</sup>. Another variation on this theme states that the present abundances of highly siderophile elements in the Earth's mantle (the late veneer) result from the addition of a small portion of the impactor's core, while the rest of the impactor's core accreted to the Earth's core without significant interaction with the Earth's mantle. The Moon shows little evidence of a late veneer of siderophile elements, supporting an event unique to the Earth as the explanation for the abundances of the highly siderophile elements in the Earth's mantle.

To obtain the observed mantle abundances of the highly siderophile elements requires adding material with chondritic abundances equal to 0.74% of the mass of the Earth's mantle<sup>55</sup>. This can be achieved with 3–4% of the impactor's core, assuming that the total mass of the impactor is between 0.12 and 0.17 Earth masses, respectively. The percentage of the impactor core required depends on the size of the impactor, and is independent of the size of the impactor core because the highly siderophile elements from the impactor would be quantitatively concentrated in the metal. The amount of metal containing the siderophile elements added to the mantle is very small: 0.2 wt% of the Earth's mantle for an impactor core size of 31 wt%, and less than that for smaller cores.

As for the moderate siderophiles, the probable differentiation of the impactor into mantle and core would have depleted these elements in the impactor mantle to levels at or below terrestrial mantle values. Thus the addition of impactor mantle amounting to <10% of the mass of the terrestrial mantle would not significantly alter the terrestrial abundances of the moderately siderophile elements. The contribution of both moderately and highly siderophile elements from the impactor's core to the abundances in the Earth mantle would be only ~1%, insignificant compared with the 5–10% levels in the Earth's mantle.



# Conclusions

From these arguments we deduce that not only the dynamics, but the chemical properties of the Earth-Moon system can be explained by a low-velocity ( $5 \text{ km s}^{-1}$ ) collision with the Earth of a body of  $\sim 0.14$  Earth masses (larger than Mars) during the final stages of the accretion of the Earth from a hierarchy of planetesimals. The impacting body was already differentiated into a metallic core and silicate mantle; the material that ended up in the Moon was derived principally from the impactor's mantle, which must have been depleted in siderophile elements by core segregation, leaving siderophile abundances somewhere between lunar and present terrestrial mantle values. The core of the impactor was  $<10\%$  of its mass, if there was equilibrium between it and the mantle, and the fraction of S-rich metal in the impactor's core was  $<50\%$  of the core's mass. The core might have been larger, if the core and mantle were not in equilibrium. The impactor's mantle had volatile-element abundances similar to those of the terrestrial planets, and was enriched in FeO compared to the Earth.

The fate of the impactor's core has important implications for the abundances of siderophile elements in the Earth's mantle. The chondritic relative abundances of the highly siderophile elements can be explained if  $\sim 3\text{--}4\%$  of the impactor's core were mixed into the Earth's mantle, the remainder of the core accreting to the Earth's core without interacting with the mantle.

For fuller substantiation, the giant impact hypothesis requires more data, some of which can be collected only on future space missions. For example, the geochemical behaviour of the elements under the extreme conditions of the impact is poorly

understood and it is not clear how much of the depletion of volatile elements and enrichment of refractory elements in the Moon is a result of the impact. To answer this, we need more information on the overall composition of the lunar surface, which could be obtained by an orbiting probe; additional data on heat flow, which would put constraints on bulk composition; and seismic measurements to determine the size of the lunar core and confirm the density discontinuity at 500 km depth. Future lunar missions might obtain direct samples of the lunar mantle from the central peak of Copernicus where mantle rock has evidently been exposed by rebound following the impact that formed the crater.

On Earth, we need to know more about the chemistry of siderophile elements in the mantle, and more about the lower mantle, which remains largely unexplored. Further from home, mechanisms of planetary accretion have to be examined. This will require an understanding of the variations in the composition of the original nebula, which can be estimated from the geochemistry of Mercury, Venus and Mars. The forthcoming exploration of the martian moon, Phobos, and probes to distinct regions of the asteroid belt may also provide insights into the formation of the now-vanished hierarchy of planetesimals that accreted to form the terrestrial planets, and of the one that provided us with our unique and poetically inspiring Moon.  $\square$

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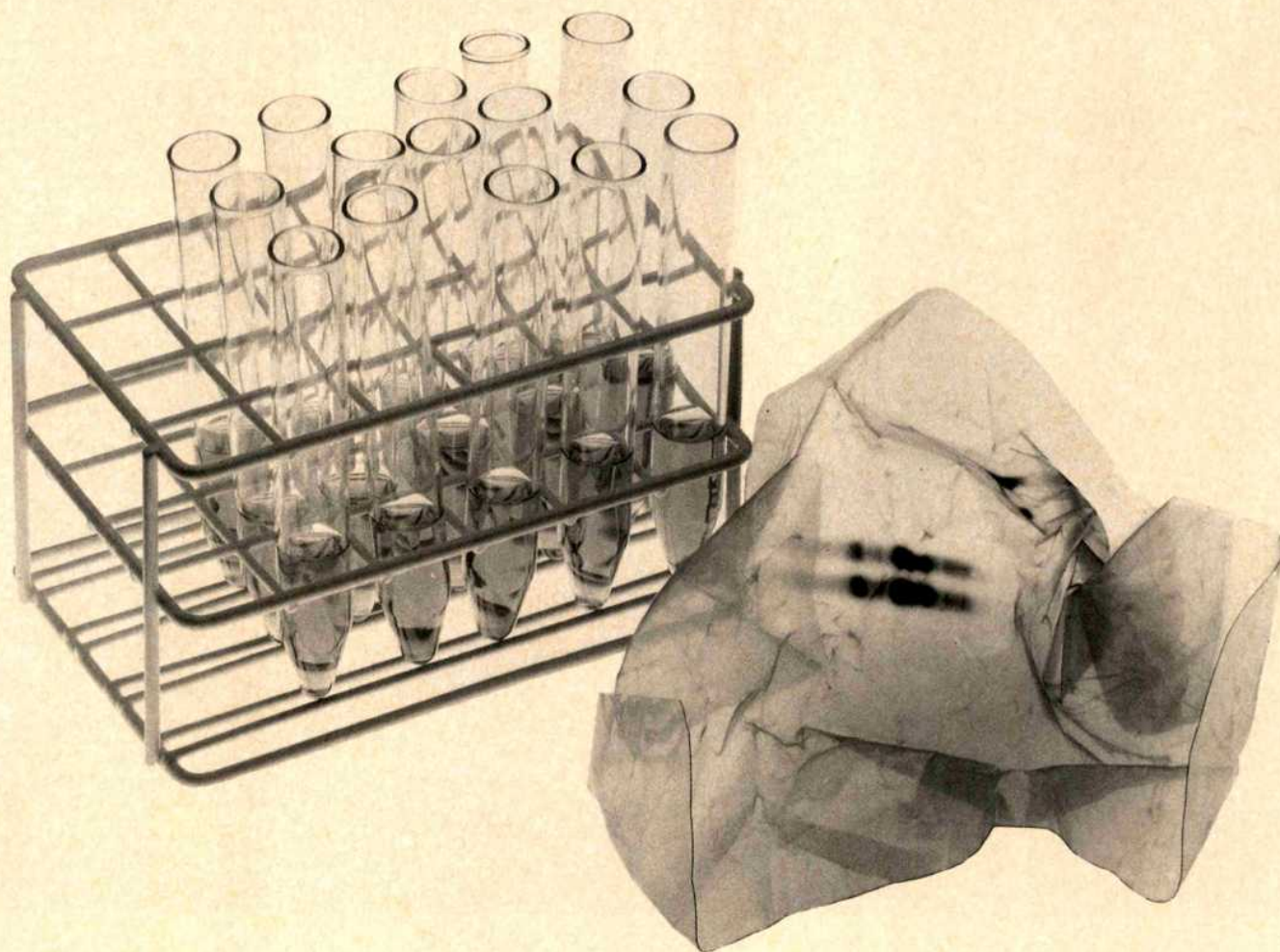
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# An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*

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An initiation site for meiotic gene conversion has been identified in the promoter region of the *ARG4* gene of *Saccharomyces cerevisiae*. The chromosome on which initiation occurs is the recipient of genetic information during gene conversion.

CONVENTIONAL genetic analysis has failed to provide a clear understanding of the mechanism of homologous recombination in meiosis. The mechanism for initiation of recombination remains particularly controversial, as most of the genetic data can be explained by models that invoke either double-strand breaks<sup>1</sup> or single-strand nicks<sup>2</sup> as the initiating DNA lesion. The existence of specific sites for the initiation of recombination has been suggested<sup>3</sup> to explain the genetic phenomenon of polarity: a gradient in the frequency of gene conversion from one end of a gene to the other (reviewed in refs 4 and 5). No such site has been identified and characterized however. Here, and in the accompanying paper<sup>6</sup>, we describe the localization and characterization of a recombination initiation site near the *ARG4* gene of the yeast *S. cerevisiae*. The properties of this site provide evidence for the double-strand-break repair model<sup>1</sup> of meiotic recombination.

We have used classical genetic analysis of deletions constructed by recombinant DNA methods to map the *ARG4* initiation site. A diploid cell that is heterozygous at a mutant site (*m*/+) usually produces two *m* (mutant) spores and two + (wild-type) spores (a mendelian segregation). In some fraction of meioses, however, gene conversion events give rise to progeny where the ratio of + to *m* is 3:1 or 1:3 (non-mendelian segregation). In these cases the information at the position of the mutation on one chromatid of a pair of sister chromatids is lost and replaced with the corresponding information from its homologue. To search for an initiation site, we first re-examined the pattern of recombination within *ARG4*, followed by a deletion analysis to identify sequences essential for high levels of meiotic gene conversion within this gene.

## Polarity of gene conversion within *ARG4*

Meiotic recombination in the *ARG4* gene has been studied extensively by Fogel and colleagues (reviewed in ref. 7). The frequency at which an *arg4*<sup>-</sup> allele engages in gene conversion depends on its position, with the frequency diminishing from one end of the gene to the other. To eliminate the possibility that the variation in conversion frequency was a result of marker effects, silent base substitutions or strain background effects, we re-examined the pattern of recombination within *ARG4* using a set of alleles constructed *in vitro*. Seven mutant alleles were constructed from cloned *ARG4* DNA and transplanted into the yeast genome at the *ARG4* locus, without the addition of any other genetic material. All seven mutations destroy recognition sites for restriction enzymes. Six are insertions or deletions of two to four base pairs (bp) (Fig. 1) and one is a G→C mutation at position +3 of the coding region.

Strains carrying each of these *arg4*<sup>-</sup> alleles were crossed with wild-type (*ARG4*<sup>+</sup>) haploids, and tetrads were dissected to determine the gene conversion frequency of each allele (Fig. 1). There is a clear gradient in gene conversion frequencies, with

5' alleles having high frequencies and 3' alleles having much lower ones. The values range from 9.1% to 0.4% of total meioses. The gradient of conversion is seen even for mutations of the same physical nature. The *Acc* and *Aha* mutations, both of which are 2-bp additions, have a fourfold difference in conversion frequency. Likewise, the *Bcl* and *Bgl* mutations, both 4-bp additions, convert at frequencies that differ by a factor of eight.

Although all the addition and deletion mutants showed only 3<sup>+</sup>:1<sup>-</sup> and 1<sup>+</sup>:3<sup>-</sup> aberrant tetrad classes, the G→C mutation was characterized by a relatively high frequency of tetrads undergoing post-meiotic segregation (reviewed in ref. 4), amounting to about 20% of all aberrant segregations.

The pattern of meiotic recombination at *ARG4* was also examined in a diploid heteroallelic for the *RV* and *Bgl* mutations, which are separated by 1,015 nucleotides. Gene conversion accounted for 88% of the intragenic recombination events, and 83% of these were single-site events that included only the *RV* site. These data are consistent with the polarity observed in single-point crosses, and allowed us to consider the frequency of gene conversion at the *RV* site as being representative of the level of genetic recombination within *ARG4*.

These studies, using *in vitro* constructed mutations, confirm the original observations of polarity within *ARG4*<sup>7</sup>, and show that this phenomenon is independent of the particular genetic background and alleles used.

## Homozygous deletions

To identify DNA sequences that are important for gene conversion within *ARG4* we used deletion analysis of a 9-kilobase (kb) region that extends from 7.5 kb upstream of the gene to a position 172 bp 3' to the *ARG4* coding region. We expected three possible deletion phenotypes, assuming that a single site was responsible for the gene conversion gradient. First, deletions upstream of the initiation site or downstream of the *ARG4* marker being tested should have no effect on gene conversion. Second, deletions that remove or inactivate the site should lower the conversion frequency within *ARG4*. Finally, deletions that remove DNA between the site and the marker being assayed should increase the conversion frequency of that marker by moving the marker closer to the initiation site.

We have used the frequency at which the *arg4-RV* allele converts to monitor the effects of 11 different homozygous deletions. The conversion frequency of this marker is not affected by deletions that lie upstream of position -316 or downstream of position +345 ( $\Delta 1$ ,  $\Delta 2$ ,  $\Delta 3$  and  $\Delta 15$ , Fig. 2a). In contrast, deletions  $\Delta 4$ ,  $\Delta 5$ ,  $\Delta 6$ ,  $\Delta 7$ ,  $\Delta 8$  and  $\Delta 9$  (Fig. 2b) all reduce the frequency of gene conversion for *arg4-RV*. The reductions, which range from two to ninefold, are all significantly lower than the wild-type level of 7.4%. All these deletions have 3' endpoints between positions -316 and +1, a region that includes all of the *ARG4* promoter<sup>8</sup>.

These deletions define a site that is required for the high frequency of gene conversion at the 5' end of the *ARG4* gene. If this site is an initiation site, deletions between this site and

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a marker should increase the conversion frequency of that marker. We have used the *arg4-Bgl* and *-Dra* mutations to look for such an effect (Fig. 3). Deletion  $\Delta 14$  removes 980 bp of DNA from the coding region and moves the *Bgl* marker almost as close to the proposed initiation site as the *RV* marker is in the wild-type gene. In the presence of this homozygous deletion, gene conversion at the *Bgl* site increases from its normal frequency, 0.4%, to 7.2%, roughly the same as that at the *RV* site in non-deletion controls. Two control crosses confirm that sequences mapping to the region defined by the deletions in Fig. 2 are responsible for this effect. One of these extends the deletion by 142 bp further 5' than  $\Delta 14$  (deletion  $\Delta 13$ ), and the second extends it 316 bp further 5' than  $\Delta 14$  (deletion  $\Delta 12$ ). Gene conversion of *arg4-Bgl* was 1.2% with  $\Delta 13$ , and 1.0%

ARG4								
	NspH	Acc	RV	Bcl	Dra	Aha	Bgl	
Mutation(bp)	G→C	+2	-2	+4	-3	+2	+4	
Position(bp)	+3	131	260	345	601	830	1,274	
3+:1-	21	22	31	13	11	1	0	
1+:3-	17	20	31	7	10	6	3	
p.m.s.	10	0	0	0	0	0	0	
Total tetrads	530	674	838	584	742	480	676	
Conversion frequency (%)	9.1	6.2	7.4	3.4	2.8	1.5	0.4	

FIG. 1 Gene conversion frequencies for *in vitro* constructed *ARG4* mutations. Seven *arg4*<sup>-</sup> alleles were constructed *in vitro* and transplanted into the *S. cerevisiae* genome at the *ARG4* locus. The mutations denoted *Acc*, *RV*, *Bcl*, *Dra*, *Aha* and *Bgl* are small insertions or deletions that destroy the recognition sites for the restriction enzymes *AccI*, *EcoRV*, *BclI*, *DraIII*, *AhaI* and *BglII* respectively. The G→C mutation at position +3 is a single base substitution that destroys an *NspH* site. Nucleotide position +1 is the first base pair in the *ARG4* coding region<sup>8</sup>. The number of conversion events in each direction is listed separately, and combined to yield the conversion frequency. Of the ten tetrads showing post-meiotic segregation (p.m.s.) for the mutation at position +3, one was 5<sup>+</sup>:3<sup>-</sup> and nine were 3<sup>+</sup>:5<sup>-</sup>.

**METHODS.** Standard methods for manipulating and modifying DNA were used to construct the *ARG4* mutations<sup>20</sup>. Plasmid p(SPO13)2 was the source of *ARG4* DNA used in these studies<sup>21</sup>. The G→C transversion mutation was generated by oligonucleotide-directed mutagenesis<sup>22</sup> using a synthetic 25-mer with a single base difference from the wild-type *ARG4* sequence at the third base of the AUG initiation codon. All of the mutated plasmids were transformed into *Escherichia coli* strain NPS/RK2 (relevant genotype *argH1 leuB6 trpC1117 pyrF dam*<sup>-</sup>). The *argH1* mutation in this strain can be complemented by the wild-type *ARG4* gene<sup>23</sup>, but not by any of the seven mutant genes. All yeast strains used were derived by lithium acetate transformation<sup>24</sup> of the isogenic strains<sup>25</sup> MGA1 (*MAT $\alpha$  leu2-3, 112 ura3-52 his3 $\Delta$ 1 trp1-289*), MGA2 (*MAT $\alpha$  ura3-52 his3 $\Delta$ 1 trp1-289 ade2*), MGA3 (*MAT $\alpha$  leu2-3, 112 ura3-52 trp1-289*) and MGA11 (*MAT $\alpha$  leu2-3, 112 ura3-52 ade2*). Cycloheximide-resistant derivatives of MGA1 and MGA2 were selected on 10  $\mu$ g ml<sup>-1</sup> cycloheximide. The *ARG4* mutations were introduced into yeast either by the standard gene-transplacement technique<sup>26</sup> or by a co-transformation procedure<sup>27</sup>. Single *arg4*<sup>-</sup> colonies from each of the seven transformants were crossed to *ARG4*<sup>+</sup> yeast, diploids were selected, sporulated and the resultant asci were dissected to determine the gene conversion frequencies for each *arg4*<sup>-</sup> allele. The segregation of *ARG4* and at least five additional heterozygous markers was examined for each cross, and only tetrads displaying aberrant segregation at a single locus were scored as conversion events. Media and growth conditions were essentially as described<sup>28</sup>. Diploid cells were pregrown to a cell density of about  $8 \times 10^7$  cells ml<sup>-1</sup> in YPD (1% yeast extract, 2% Bacto-peptone, 2% dextrose), washed once in water and transferred to liquid sporulation medium at a cell density of  $2 \times 10^7$  cells ml<sup>-1</sup>. Sporulation was carried out at 30 °C with vigorous shaking in 1% potassium acetate, 0.1% Bacto-yeast extract, 0.05% dextrose plus any nutritional supplement required by the particular diploid. Spore viability typically >95%.

with  $\Delta 12$ ; these values are not significantly different from each other or from the value of 0.4% reported for the *Bgl* site in Fig. 1. These results indicate that the conversion frequency of *arg4-Bgl* can be increased by a factor of at least six by moving it closer to the conversion element (comparing  $\Delta 13$  with  $\Delta 14$ ), and that sequences between -139 and +3 are essential for the high frequency of gene conversion. In a similar experiment, we used *arg4-Dra*, which converts at a frequency of 2.8%. Upon deletion of 566 bp of DNA between positions -37 and +529 ( $\Delta 11$ ), the frequency of gene conversion at the *Dra* site increased to 6.2%, an increase of more than twofold over the wild-type frequency at this site, and a value that is not significantly different from the wild-type frequency for *arg4-RV*. The results of these internal deletion studies indicate that most, if not all of the conversion element is 5' to position -37, as both  $\Delta 14$  and  $\Delta 11$  can raise the frequency at which downstream alleles convert to a level indistinguishable from the frequency determined for *arg4-RV*. These results confirm the notion that the gradient in gene conversion frequency reflects the physical distance between a marker and the initiation site and not marker effects.

The above experiments place the 5' and 3' boundaries of the sequences that stimulate gene conversion between positions -316 and -37. We were therefore surprised that deletion  $\Delta 10$  (-316 to +3) did not affect conversion at the *RV* site (Fig. 2b). As ten deletions that remove all or part of this region have significant effects on gene conversion, we suspect that in the  $\Delta 10$  deletion, two sequences have been fused together to create a new sequence that can function to initiate gene conversion.

### Direction of information transfer

Deletion of an initiation site from one homologue should lead to a bias in the direction of information transfer (disparity) as only the wild-type chromosome can initiate gene conversion. In a cross that is heterozygous for the 142-bp deletion  $\Delta 9$  ( $\Delta 9$ , Fig. 2b; cross D9HET, Fig. 4), there is a fourfold excess of tetrads that are 3 $\Delta$ :1+ over those that are 1 $\Delta$ :3+, indicating that the wild-type DNA sequence that lies opposite the deletion is the preferred recipient of information in gene conversion. About half (562) of the tetrads reported for this cross were also heterozygous for *arg4-RV*. Six aberrant segregations at this site were observed, and all six were co-converted with the 142-bp heterology. An extra 10 conversion events involved only the deletion and did not include the *RV* site. These observations indicate that conversion events originate in or near the sequences removed by deletion  $\Delta 9$ .

A similar cross between two haploids carrying different deletions results in an 84-bp heterozygosity which includes sequences necessary for the expression of *ARG4* (cross  $\Delta 5/\Delta 6$ , Fig. 4). There is a 22:1 bias in the direction of information transfer in this cross, with the homologue that retains the sequences from -140 to -56 acting as the preferred recipient in gene conversion. We used two control crosses to show that not all insertion/deletion heterozygosities show this disparity (Fig. 4). One cross had a 137-bp insertion, and the other an 84-bp deletion in the *ARG4* coding sequence. Both of these heterozygosities fall within regions of the gene that are, on the basis of our deletion analysis, downstream of the initiation site. Both heterologies show a modest tendency to convert towards the deletion, or the shorter, of the two recombining *ARG4* regions, but in neither case is the ratio significantly different from an expected ratio of one. It seems that strong disparity is a property only of heterozygous deletions involving the initiation site.

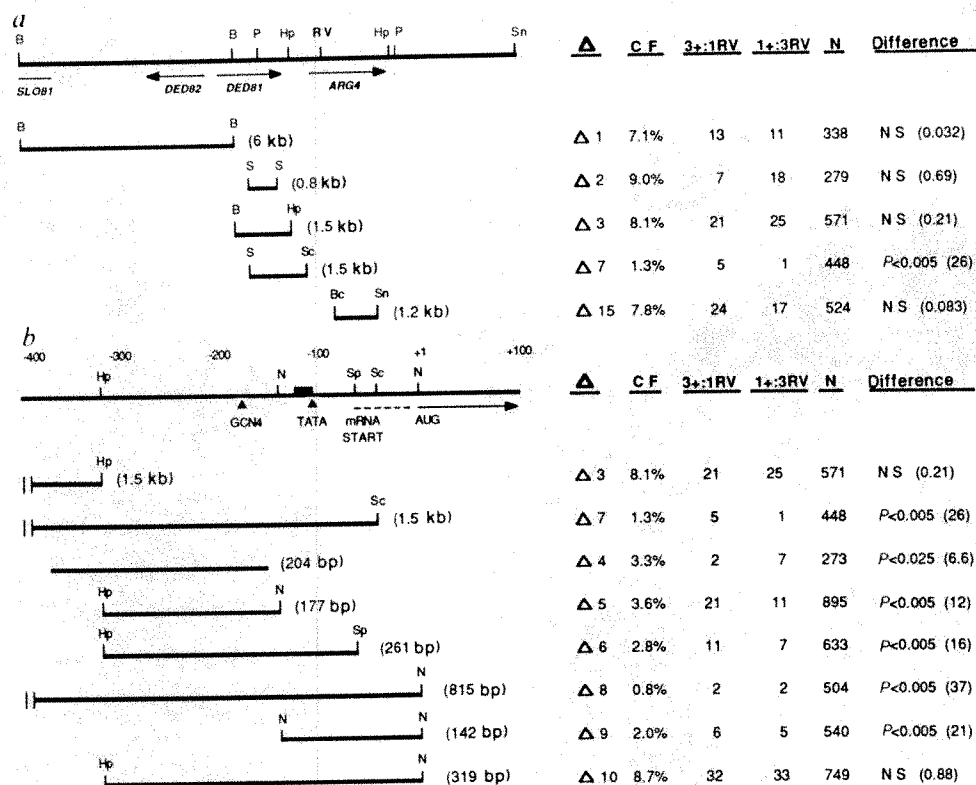
### Discussion

Several lines of evidence indicate that a region overlapping the *ARG4* promoter acts as an initiation site for meiotic recombination. First, deletion of the site abolishes the high frequencies of gene conversion seen at the 5' end of the *ARG4* gene. Second, deletions that move the site closer to a marker raise the frequency at which that marker engages in gene conversion. Third,

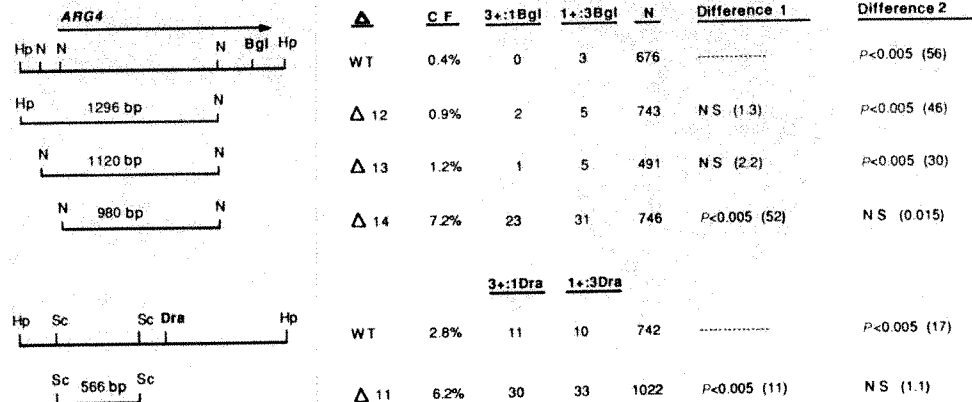
**FIG. 2** Effects of homozygous deletions on meiotic gene conversion at the *RV* site of *ARG4*. Solid black bars represent the DNA sequences deleted in each of the 11 crosses assayed. Positions of deletion breakpoints are numbered from the first base in the AUG codon of *ARG4* (position +1). The number of conversion events in each direction is listed separately, and combined to yield the conversion frequency (CF). *N* is the number of four-spore viable tetrads analysed; + indicates wild-type information; *RV* indicates the mutant site. The column labelled 'difference' indicates whether the conversion frequency is significantly different from the value of 7.4% determined for the *RV* site (Fig. 1). The level of significance is indicated by the *P*-value. Also listed are the *G*-values (parentheses) that were used in the significance testing (calculated using the *G*-test of independence<sup>29</sup>). NS, no significant difference. B, *Bam*HI; Bc, *Bcl*I; Hp, *Hpa*I; N, *Nsp*HI; P, *Pst*I; RV, *Eco*RV; S, *Sal*I; Sc, *Sac*I; Sn, *Sna*BI; Sp, *Ssp*I. a, A 13.5-kb region surrounding the *ARG4* gene and a partial restriction map is shown. Arrows indicate the directions of transcription for the *DED81*, *DED82* and *SLO81* genes. b, A 700-bp region around the 5' end of the *ARG4* gene is shown. The 3' endpoints of  $\Delta 3$  and  $\Delta 7$  are shown for orientation purposes. The positions of the experimentally determined *GCN4*-binding site<sup>30</sup>, a possible TATA element, and the measured mRNA start site are indicated. The black box represents a run of 14 A residues (*A*<sub>14</sub>). All of these elements have been proposed to be involved in transcription of *ARG4*<sup>31</sup>.

**METHODS.** Deletions (except  $\Delta 4$  and  $\Delta 10$ ) were constructed by cutting with the restriction enzymes indicated.  $\Delta 4$  is a spontaneous deletion of 204 bp that arose by selecting for *arg*<sup>+</sup> function in *E. coli*<sup>23</sup>.  $\Delta 10$  was constructed by oligonucleotide-directed mutagenesis<sup>22</sup> using a synthetic 48-mer that spans the deletion breakpoints. Plasmids containing these deletions were subjected to DNA sequence analysis to ensure that no additional sequences were lost or added. This analysis revealed that  $\Delta 8$  extended 55 bp further upstream than the *Sal*I site at position -0.76 kb. Haploid strains were constructed with the *URA3* gene<sup>32</sup> inserted at various positions within and upstream of *ARG4*. Deletions were introduced by co-transformation<sup>27</sup> with a CEN plasmid followed by screening for loss of the *ura*<sup>+</sup> phenotype, and then by Southern analysis for the presence of the deletion. Genetic analysis of the *URA3* inserts revealed two essential genes upstream of *ARG4*, *DED81* and *DED82*, and one gene, *SLO81* which results in an extremely slow growth

**FIG. 3** Effects of homozygous deletions on meiotic gene conversion at the *Bgl*I (deletions  $\Delta 12$ ,  $\Delta 13$  and  $\Delta 14$ ) and *Dra* sites ( $\Delta 11$ ) in *ARG4*. Solid black bars represent DNA sequences deleted in each of the experimental crosses. The numbers of conversion events in each direction are listed separately and are combined to yield the conversion frequency. The data for wild-type (WT) levels of conversion at these sites are taken from Fig. 1. The column headed 'difference 1' indicates whether the conversion frequency is significantly different from the wild-type level at that particular site (data from Fig. 1). + indicates wild-type information; *Bgl*I and *Dra*I indicate the mutant sites. The column headed 'difference 2' indicates whether the conversion frequency is significantly different from the wild-type frequency at the *RV* site (data from Fig. 1). Abbreviations and statistical methods are as in Fig. 2. Because deletions  $\Delta 11$ ,  $\Delta 12$ ,  $\Delta 13$  and  $\Delta 14$  confer an *arg*<sup>-</sup> phenotype, *ARG4*



phenotype. All diploids used in the deletion analysis contain a 10.5 kb insert in the *URA3* gene at its normal chromosomal position<sup>33</sup>. This insert, designated *dup*<sup>+</sup>, contains sequences from -7.3 kb to +5.2 kb around *ARG4*, but without a 2-kb fragment from -316 bp to +1,745 bp that carries the *ARG4* gene<sup>8</sup>. The *dup*<sup>+</sup> construct complements deficiencies created upon disruption of *DED81* or *DED82*. Yeast transformation and tetrad analysis were as described in the Fig. 1 legend. Deletions  $\Delta 6$  through  $\Delta 10$ , and  $\Delta 15$  confer an *arg*<sup>-</sup> phenotype to yeast cells. The *ARG4* genotype of meiotic progeny was determined by allele testing, using a pair of tester strains carrying the *arg4-RV* mutation. Original dissection plates were printed onto a lawn of cells that were *arg4-RV*, *thr4*<sup>-</sup> and either *MATa* or *MATα*. After mating for 16 h at 30 °C, the plates were printed onto minimal media and irradiated with a 354 nm UV light source for 1 min at distance of 1 m. Cells that are *arg4-RV* cannot give rise to *arg*<sup>+</sup> papillae by recombination with an *arg4-RV* tester. All tetrads showing non-mendelian segregation were retested with an *arg4-RV* and other *arg4*<sup>-</sup> testers to ensure that *arg4*<sup>-</sup> allele was not generated by ectopic recombination with the *dup*<sup>+</sup> locus at *URA3* on chromosome V. Such events constitute ≤0.5% of all gene conversion events at *ARG4* (data not shown).



genotypes were scored as described in the Fig. 2 legend, using *arg4-Bgl* or *arg4-Dra* tester strains. For  $\Delta 11$  and  $\Delta 14$ , the data are pooled from crosses with and without *dup*<sup>+</sup> homozygous. The *dup*<sup>+</sup> genotype had no significant effect on the gene conversion frequency (data not shown).



heterozygous deletions show disparity of gene conversion, as expected when initiation occurs preferentially on one chromosome. Fourth, the initiation site is co-converted with the markers upon which it acts, which is consistent with recombination events starting at the initiation site and extending for variable distances away from the site. Finally, as we show in the accompanying paper<sup>6</sup>, we are able to detect the transient appearance of double-strand breaks at the initiation site during meiosis.

The property of disparity is important in considering models for initiation. We find that the chromosome on which events are initiated is the recipient of information in gene conversion. This is consistent with studies on meiotic hotspots in other fungi (for example, the *M26* mutation in the *ADE6* gene of *Schizosaccharomyces pombe*<sup>9</sup>, the *YS17* mutation in the *BUFF* locus of *Sordaria brevicollis*<sup>10</sup>, and the *cog*<sup>+</sup> variant near the *HIS3* gene of *Neurospora crassa*<sup>11</sup>). It has also recently been shown<sup>12</sup> that a yeast centromere can act to inhibit adjacent sequences from acting as recipients, but not donors, of information during meiotic gene conversion. The simplest explanation for this phenomenon is that the centromere acts in *cis* to inhibit initiation.

It therefore seems to be a general phenomenon that the chromosome on which initiation occurs is the recipient of information in gene conversion. This is inconsistent with the original Aviemore model<sup>2</sup> for recombination, in which a single-strand displaced from the initiating chromosome is transferred to the recipient chromosome. It is, however, consistent with either the modified Radding model<sup>13</sup>, in which a single-stranded gap on the initiating chromosome is repaired using information from the intact donor chromosome, or with the double-strand-break repair model<sup>1</sup>, in which conversion occurs by repair of a double-strand gap on the initiating chromosome using information on the intact donor chromosome.

The inevitable evolutionary consequence of a system in which initiation sites are the preferred recipients in gene conversion is that the sequences will tend to be lost as a result of the event itself. It is paradoxical that sequences that stimulate recombination are lost as a mechanistic consequence of this process, especially as studies on chromosome segregation in meiosis I have shown that recombination is essential for proper chromosome disjunction (reviewed in ref. 14). A possible solution to this paradox is that by coupling the signals for recombination to those for a cellular process that is under strong selective

pressure (for example transcription), any loss of initiation sites for recombination that may occur is compensated for by selection to maintain functional promoters.

The region that we have identified as the initiation site overlaps with the *ARG4* promoter. *ARG4* transcripts increase by 5- to 10-fold within one hour of transfer to sporulation medium (data not shown). Deletions that remove sequences essential for transcription of the *ARG4* gene and give rise to an *arg*<sup>-</sup> phenotype are highly deficient in *ARG4* transcripts in meiosis and show decreased conversion. With the exception of  $\Delta 10$ , which we believe creates a cryptic initiation site, no deletion that destroys any part of the promoter is without an effect on gene conversion.

In *S. cerevisiae*, a relationship between transcription and recombination has been demonstrated for mating-type switching<sup>15</sup> and for the *HOT1* site<sup>16</sup>, a sequence that stimulates mitotic recombination and consists of the essential sequences for ribosomal DNA transcription. Mitotic recombination between duplicated *GAL10* genes has recently been shown to be strongly stimulated by galactose-induced transcription (B. Thomas and R. Rothstein, personal communication). At present, however, we cannot eliminate the possibility that the *ARG4* promoter and conversion element merely overlap, or that the conversion element is a specific DNA sequence that stimulates recombination in the context of an active promoter.

It is important to note that gene conversion is decreased, but not abolished, in the absence of the initiation site that we have identified. The deletions that have the strongest effects,  $\Delta 7$ ,  $\Delta 8$ , and  $\Delta 9$ , still allow 1-2% gene conversion at the *RV* site. This background of gene conversion is probably due to initiation at other sites.

One of the major unanswered questions about meiotic recombination is to what extent heteroduplex DNA contributes to gene conversion. In single-strand initiation models<sup>2,13</sup>, all conversion occurs by heteroduplex formation followed by mismatch repair. In the double-strand-break repair model<sup>1</sup>, gene conversion can occur either by double-strand gap repair or by mismatch repair of heteroduplex DNA. Some heteroduplex DNA is known to form, as post-meiotic segregation is a well-documented phenomenon (reviewed in refs 4 and 5). Studies of post-meiotic segregation suggest that a minimum of 20-30% of all gene conversion events (and perhaps twice as many) between positions +3 and +341 go through a heteroduplex intermediate<sup>17</sup>. The suggestion that long regions of heteroduplex DNA can form

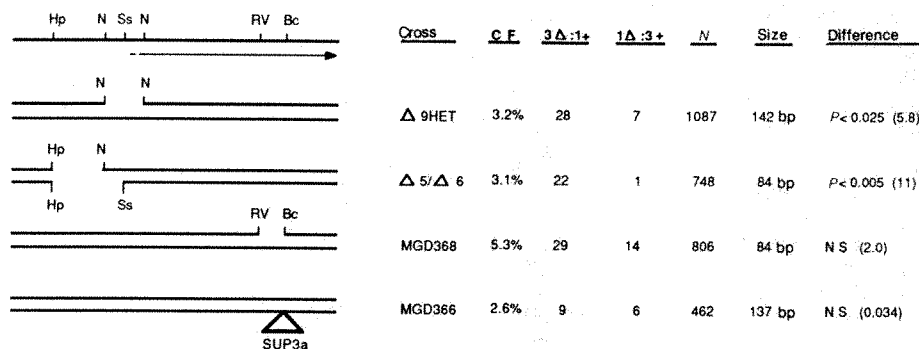


FIG. 4 Gene conversion of heterozygous deletions/insertions at *ARG4*. Data for  $\Delta 9$ HET is combined data from two similar crosses (MGD324 and MGD184), one of which (MGD324) was also heterozygous for *arg-RV* in *trans* to the deletion (see text). Both crosses displayed a similar bias in directionality of conversion for the 142 bp deletion (13 3Δ:1<sup>+</sup> and 3 1Δ:3<sup>+</sup> in MGD324 and 15 3Δ:1<sup>+</sup> and 4 1Δ:3<sup>+</sup> in MGD184). Cross  $\Delta 5/\Delta 6$  is described in the text. Conversion events in each direction are listed separately, but are combined to yield the frequency at which the heterology engages in gene conversion. Definitions and abbreviations are as in the Fig. 2 legend.  $\Delta$  indicates the deletion genotype, + denotes the wild-type configuration. The column headed

'difference' indicates the significance of the deviation from an expected ratio of one for the two conversion classes.

METHODS. Abbreviations and statistical methods are as described in the legend to Fig. 2, except that Yates' correction for small sample size was used in the calculation of  $G_{adj}^{29}$ . The 84-bp deletion that is heterozygous in MGD368 was created by digesting *ARG4* DNA with *EcoRV* and *BclI*, filling in the overhanging ends with T4 DNA polymerase and religating. The *SUP3a* fragment used in cross MGD366, a kind gift from Dr R. Rothstein, was inserted as a 137-bp *BamHI* fragment into the *BclI* site of *ARG4*. Yeast co-transformation and genetic analysis was as described in the Fig. 2 legend.

next to a region of double-strand-gap-repair is supported by the finding of extensive single-stranded DNA next to a double-strand break at the *ARG4* initiation site<sup>6</sup>, and by genetic analysis of linear and gapped plasmid DNA after transformation into yeast<sup>18,19</sup>.

The identification of a naturally occurring initiation site for gene conversion is a first step in the molecular analysis of meiotic

recombination. The further characterization of this sequence within and outside its normal context will contribute to our understanding of how initiation is regulated. By focusing on this DNA sequence throughout meiosis, intermediates in the initiation event can be identified and characterized, allowing us to establish the molecular nature of initiation and distinguish experimentally between the current models for recombination.

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# Transcription activation by the adenovirus E1a protein

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The adenovirus E1a protein stimulates transcription of a wide variety of viral and cellular genes. It is shown here that E1a has the two functions characteristic of a typical cellular activator: one direct E1a to the promoter, perhaps by interacting with a DNA-bound protein, and the other, an activating region, enables the bound activator to stimulate transcription.

RECENT experiments have defined two functional regions of cellular transcription activators<sup>1–3</sup>. One, the 'binding region', directs the protein to DNA, enabling the other, the 'activating region', to interact with a component of the transcription complex to stimulate transcription. These regions are modular: each is a separable, functional unit that can be joined to the complementary region of an unrelated activator to create a functional hybrid activator<sup>1</sup> (reviewed in ref. 3). In most instances the binding region interacts directly with a specific DNA sequence, but in some cases the binding region recognizes a DNA-bound protein<sup>4–10</sup>. The binding region determines the activator's specificity by targeting the protein to only those promoters that have the appropriate binding site. The activating regions are short acidic fragments of no specific primary sequence<sup>11–14</sup>.

The adenovirus E1a protein (E1a) is a powerful transcription activator that is required for the expression of viral early genes<sup>15,16</sup>. Several lines of evidence suggest that E1a is atypical. First, E1a stimulates transcription of a wide variety of viral and cellular genes that lack a specific promoter element required for induction. Second, E1a enhances expression of genes transcribed by both RNA polymerase II and III (reviewed in ref. 17). Third, E1a does not bind a specific DNA sequence<sup>18</sup>. These differences suggest that E1a functions by a novel mechanism. It has been proposed, for example, that E1a stimulates transcription indirectly by converting cellular transcription factors from

an inactive to an active form<sup>19–22</sup>. Despite these apparent differences we present evidence here that E1a functions like a typical cellular activator.

## DNA-bound E1a activates transcription

Previous studies have shown that a small, highly conserved E1a region (designated region 3, amino acids 140–189; see ref. 23 and Fig. 6b) is necessary, and in some instances sufficient, to activate transcription<sup>24–30</sup>. Figure 1 shows that an E1a fragment containing region 3 activates transcription in mammalian cells when bound artificially to the promoter through the DNA-binding region of another activator. For this experiment, we constructed a plasmid that expresses E1a amino acids 121–223 fused to the GAL4 DNA-binding domain, GAL4(1–147). GAL4-E1a contains the GAL4 sequences required to recognize the GAL4-binding site, but lacks the GAL4 sequences that enable promoter-bound GAL4 to activate transcription *in vivo*<sup>11,12,31–36</sup>. The activity of GAL4-E1a is measured following cotransfection with a 'reporter' plasmid containing the chloramphenicol acetyltransferase (CAT) gene linked to a promoter, with or without inserted GAL4 sites.

Figure 1a shows that GAL4-E1a activates the adenovirus E4 promoter, which lacks GAL4 sites, but does so more efficiently if GAL4 sites are present (Fig. 1a, GAL4-E1a; compare E4 and GAL4/E4 promoters). As expected, wild-type E1a, which lacks



the GAL4 DNA-binding domain, activates the E4 and GAL4/E4 promoters comparably (Fig. 1a, E1a; compare E4 and GAL4/E4 promoters). Binding GAL4(1-147) alone does not enhance activation, because GAL4(1-147) lacks an activating region<sup>11</sup> (Fig. 1a).

Insertion of GAL4 sites dramatically increases activation of two other promoters. The mouse mammary tumour virus (MMTV) promoter is activated weakly by GAL4-E1a but the

insertion of GAL4 sites increases activation at least 100-fold (Fig. 1b). We also tested a 'core' promoter, created by inserting a double-stranded oligonucleotide bearing the adenovirus E1b TATA box upstream of the CAT gene. This E1bTATA promoter is not activated detectably by wild-type E1a (data not shown) and activation by GAL4-E1a is detectable only if GAL4 sites are present (Fig. 1c). (We note that weak activation of the E1b TATA box has been observed in virus infection experiments<sup>37</sup>).

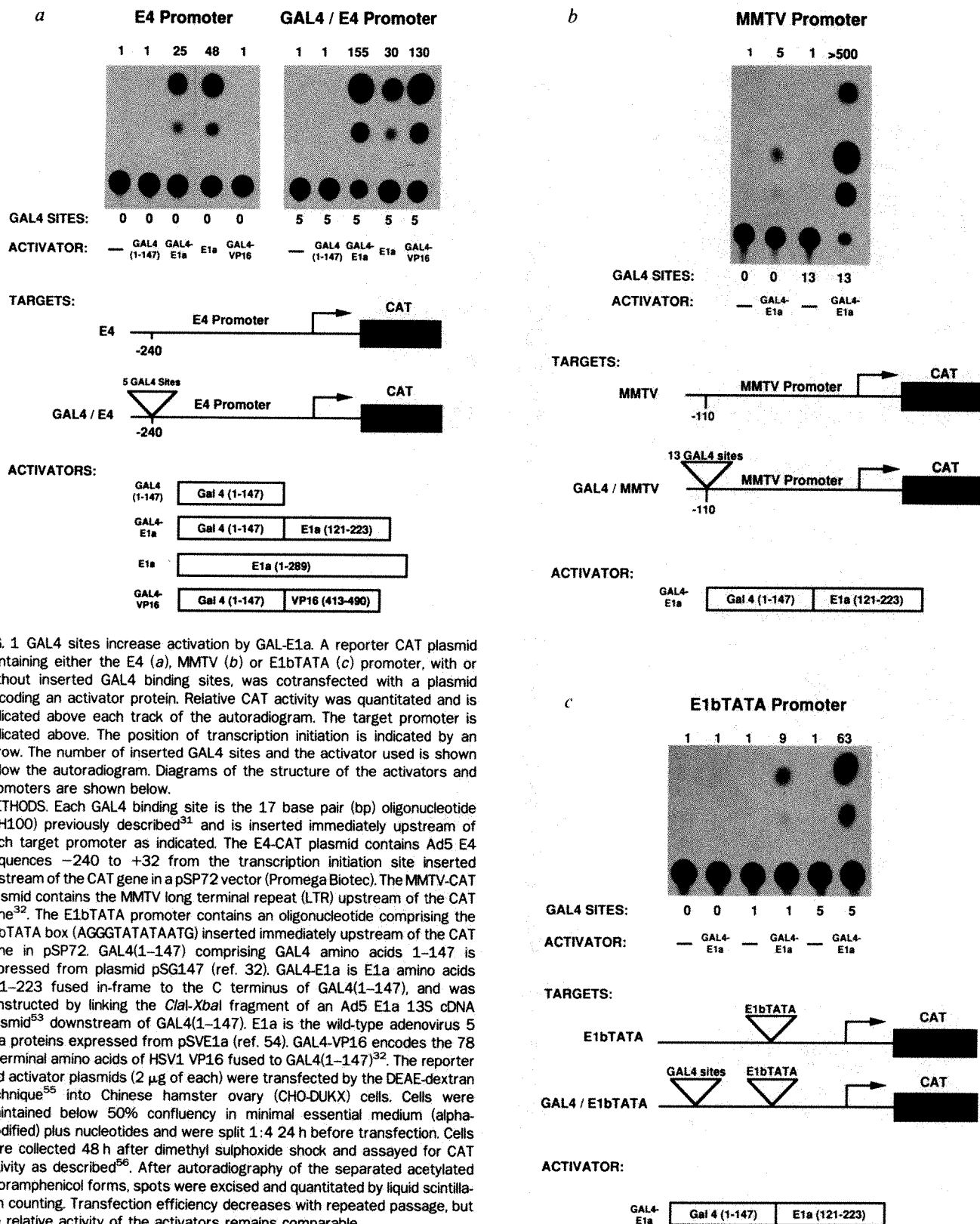


FIG. 1 GAL4 sites increase activation by GAL-E1a. A reporter CAT plasmid containing either the E4 (a), MMTV (b) or E1bTATA (c) promoter, with or without inserted GAL4 binding sites, was cotransfected with a plasmid encoding an activator protein. Relative CAT activity was quantitated and is indicated above each track of the autoradiogram. The target promoter is indicated above. The position of transcription initiation is indicated by an arrow. The number of inserted GAL4 sites and the activator used is shown below the autoradiogram. Diagrams of the structure of the activators and promoters are shown below.

**METHODS.** Each GAL4 binding site is the 17 base pair (bp) oligonucleotide (MH100) previously described<sup>31</sup> and is inserted immediately upstream of each target promoter as indicated. The E4-CAT plasmid contains Ad5 E4 sequences -240 to +32 from the transcription initiation site inserted upstream of the CAT gene in a pSP72 vector (Promega Biotec). The MMTV-CAT plasmid contains the MMTV long terminal repeat (LTR) upstream of the CAT gene<sup>32</sup>. The E1bTATA promoter contains an oligonucleotide comprising the E1bTATA box (AGGGTATATAATG) inserted immediately upstream of the CAT gene in pSP72. GAL4(1-147) comprising GAL4 amino acids 1-147 is expressed from plasmid pSG147 (ref. 32). GAL4-E1a is E1a amino acids 121-223 fused in-frame to the C terminus of GAL4(1-147), and was constructed by linking the *Cla*I-*Xba*I fragment of an Ad5 E1a 13S cDNA plasmid<sup>53</sup> downstream of GAL4(1-147). E1a is the wild-type adenovirus 5 E1a proteins expressed from pSVE1a (ref. 54). GAL4-VP16 encodes the 78 C-terminal amino acids of HSV1 VP16 fused to GAL4(1-147)<sup>32</sup>. The reporter and activator plasmids (2 µg of each) were transfected by the DEAE-dextran technique<sup>55</sup> into Chinese hamster ovary (CHO-DUKX) cells. Cells were maintained below 50% confluency in minimal essential medium (alpha-modified) plus nucleotides and were split 1:4 24 h before transfection. Cells were collected 48 h after dimethyl sulphoxide shock and assayed for CAT activity as described<sup>56</sup>. After autoradiography of the separated acetylated chloramphenicol forms, spots were excised and quantitated by liquid scintillation counting. Transfection efficiency decreases with repeated passage, but the relative activity of the activators remains comparable.

Although activation of the E1bTATA promoter is enhanced by a single GAL4 site, the presence of additional GAL4 sites amplifies the response—presumably by increasing the number of bound GAL4-E1a molecules (Fig. 1c). Taken together, these results indicate that binding E1a to DNA (or binding E1a more efficiently to DNA) enhances its ability to activate transcription. This in turn indicates that E1a may function naturally in the vicinity of the promoter.

### E1a contains an activating region

Figures 2, 3 and 4 show that E1a contains a powerful activating region required for the activation of promoters that contain GAL4 sites and those that lack them. Deletion analysis shows that E1a amino acids 139–149 (see Fig. 6c) are essential for the activity of GAL4-E1a. In these experiments, we compare the activity of several N-terminal deletion mutants of the E1a fragment 121–223. As the stability of similar proteins with different

N-terminal sequences varies greatly<sup>38,39</sup>, and as large variations in protein stability would distort comparisons, each derivative used in these and subsequent experiments begins with the same 147 amino acids, GAL4(1–147), even when the target promoter lacks GAL4 sites.

Removal of E1a amino acids 140–185 (most of region 3) abolishes GAL4-E1a's activity, whether or not GAL4 sites are present (Fig. 2a, GAL4-E1a $\Delta$ 3) even when 10 times more DNA is transfected (data not shown). In contrast, deletion of E1a amino acids 121–132 (most of region 2; see ref. 23 and Fig. 6b) does not affect GAL4-E1a's activity on either promoter (Fig. 2b, GAL4-E1a $\Delta$ N132). Deletion of the remaining six amino acids of region 2 contiguous to region 3 reduces activation slightly (Fig. 2b, GAL4-E1a $\Delta$ N138). Further deletion of the first 10 amino acids of region 3 abolishes GAL4-E1a's activity on both promoters (Fig. 2b, GAL4-E1a $\Delta$ N149), even when 10 times more DNA is transfected (data not shown). These results indi-

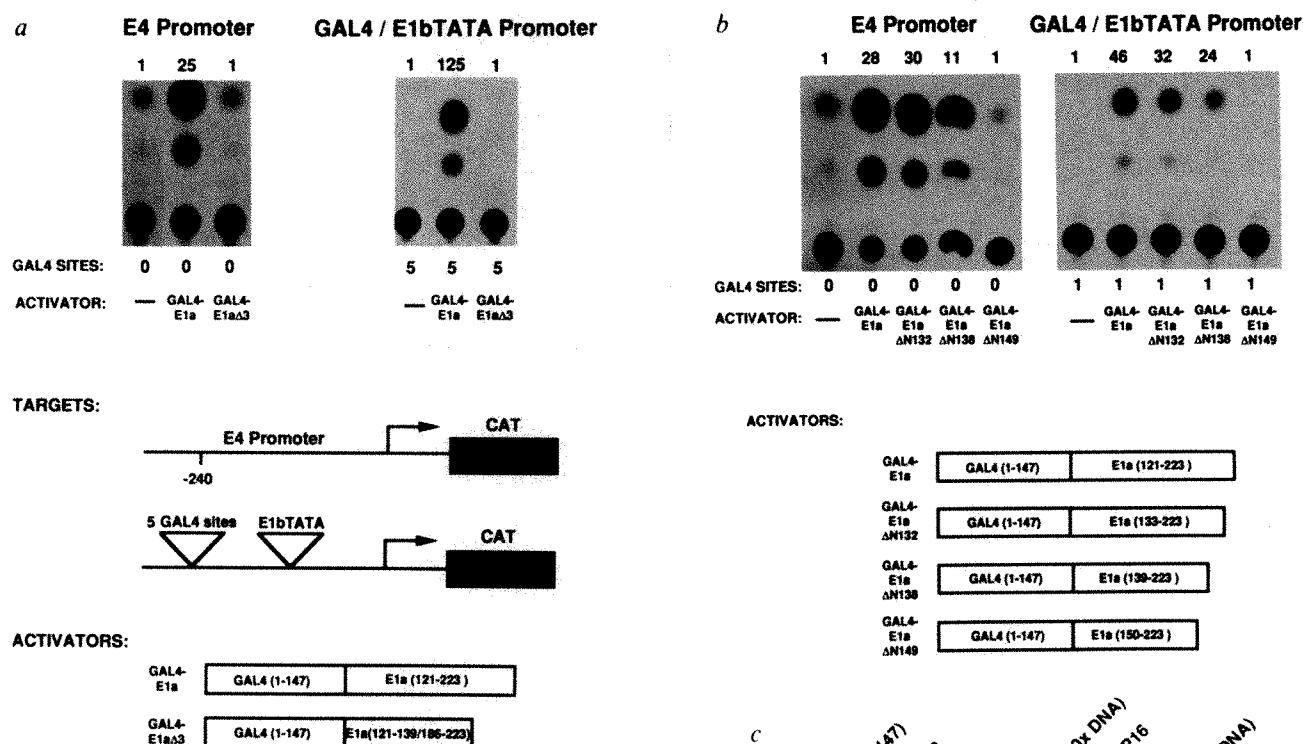
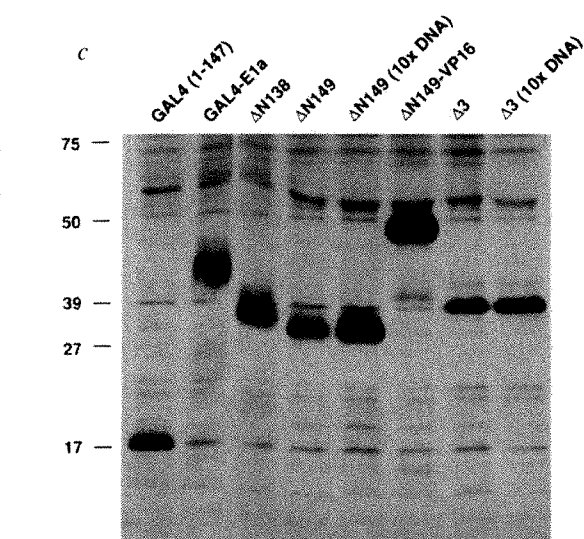


FIG. 2 E1a amino acids 139–149 are essential for activation of promoters with and without GAL4 sites. *a*, *b*. A reporter CAT plasmid containing the E4 or GAL4/E1bTATA promoter was cotransfected with a plasmid encoding the activator indicated below each track of the autoradiogram. Relative CAT activity was quantitated and is indicated above each track. *c*. Protein levels of GAL4-E1a derivatives. <sup>35</sup>S-labelled GAL4-E1a derivatives from transfected COS cells were immunoprecipitated with an anti-GAL4(1–147) antibody (a gift of I. Sadowski) and fractionated on a 10% SDS-polyacrylamide gel. The transfected activator is indicated above each lane. Relative molecular mass markers are indicated on the left.

**METHODS.** Transfections were performed as described in the Fig. 1 legend. Each activator is a derivative of GAL4-E1a. GAL4-E1a $\Delta$ 3 lacks the 13S-specific amino acids of region 3, amino acids 140–185 (see Fig. 6b), and was constructed by replacing the *Clal*-*Xba*I fragment of GAL4-E1a with the *Clal*-*Xba*I fragment of an Ad5 12S cDNA plasmid<sup>53</sup>. GAL4-E1a $\Delta$ N132 and GAL4-E1a $\Delta$ N138 are progressive 5' *Bal*/31 deletions of the 13S cDNA *Clal*-*Xba*I fragment, fused in-frame to GAL4(1–147).  $\Delta$ N132 begins at Ad5 nucleotide 956, E1a amino acid 133;  $\Delta$ N138 begins at Ad5 nucleotide 973, amino acid 139. GAL4-E1a $\Delta$ N149 contains the *Sma*I-*Xba*I fragment of the 13S cDNA plasmid fused to GAL4(1–147) and begins at Ad5 nucleotide 1010, amino acid 150. COS cells transfected using the DEAE-dextran procedure were labelled with 200  $\mu$ Ci ml<sup>-1</sup> of [<sup>35</sup>S]methionine for 3 h, 72 h after the dimethyl sulphoxide shock. Subsequently, whole-cell lysates were cleared with preimmune serum and *Staphylococcus aureus* protein A, the anti-GAL4(1–147) antibody added, and the immune complexes purified and fractionated



on a 10% SDS-polyacrylamide gel. In parallel, unlabelled whole-cell lysates prepared from COS cells cotransfected with a plasmid encoding each activator and the GAL4-E1bTATA reporter CAT plasmid were assayed for CAT activity. The results obtained in COS cells were identical to those obtained in CHO cells (data not shown).



cate that E1a amino acids 139–149 (region 3) are essential for GAL4-E1a's activity on promoters containing or lacking GAL4 sites.

To confirm that the above results reflect differences in activity of the GAL4-E1a derivatives, and not variations in protein concentration, we measured the level of each activator after transfection. Figure 2c shows that the various GAL4-E1a derivatives are expressed comparably and previous studies have shown that proteins of this size will enter the nucleus by passive diffusion<sup>40–41</sup>.

Figure 3 demonstrates that E1a amino acids 139–149 can be replaced by the acidic activating region of an unrelated activator. The herpes simplex virus 1 (HSV1) VP16 protein is a typical activator in that it contains a promoter-binding function and an acidic activating region<sup>42</sup>. The VP16 activating region is particularly powerful<sup>32</sup>. Fusion of VP16's activating region to the C terminus of GAL4-E1aΔN149 restores its ability to activate the E4 promoter (Fig. 3, compare GAL4-E1aΔN149 and GAL4-E1aΔN149-VP16). (GAL4-E1aΔN149-VP16 is expressed at a higher level than GAL4-E1aΔN149, Fig. 2c, but this is not sufficient to account for the difference in activity.) Thus, the acidic activating region of the HSV1 VP16 protein can substitute for the natural activity of E1a amino acids 139–149 (as assayed on the E4 promoter), although these two protein fragments bear no obvious sequence similarity. We conclude that this portion of E1a functions solely as an activating region. This experiment is in essence a 'domain swap'<sup>1,13,32,33,43–45</sup>.

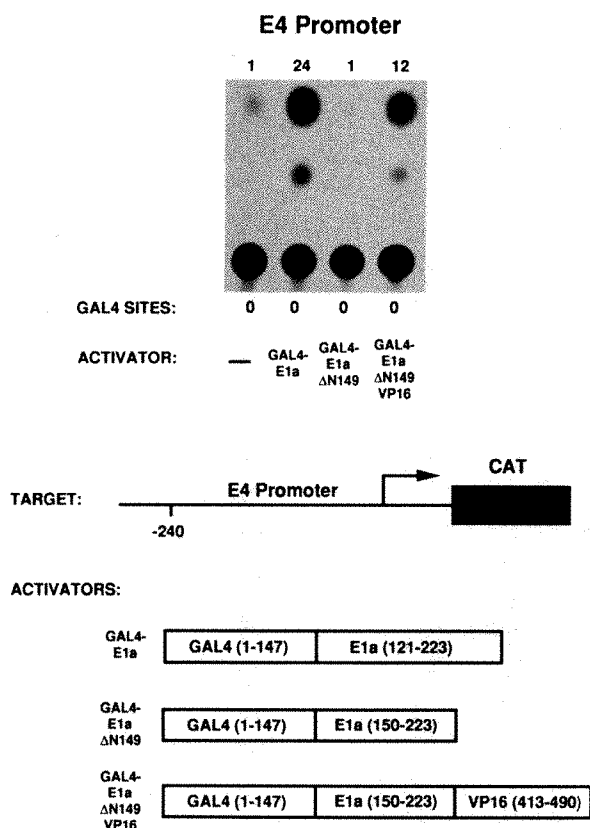


FIG. 3 E1a amino acids 139–149 function as an activating region. A reporter CAT plasmid containing the E4 promoter was cotransfected with a plasmid encoding the activator indicated below each track. Relative CAT activity was quantitated and is indicated above each track.

METHODS. Transfections were performed as described in the Fig. 1 legend. GAL4-E1a and GAL4-E1aΔN149 are described in the legends to Figs 1 and 2, respectively. GAL4-E1aΔN149-VP16 contains the 78 C-terminal amino acids of the HSV1 VP16 protein fused in-frame to the C terminus of GAL4-E1aΔN149.

Finally, GAL4-E1a is a powerful activator, consistent with the notion that E1a, a potent activator, contains a strong activating region. The GAL4/E1bTATA promoter allows us to gauge the strength of E1a's activating region, as transcription is not detectable in the absence of GAL4 sites (Fig. 4). In this assay, E1a's activating region is at least 20 times stronger than the activating regions of either wild-type GAL4 or the GAL4 derivative, GAL4-B17 (Fig. 4, GAL4, GAL4-B17; ref. 12). In fact, the activity of GAL4-E1a is only twofold less than that of GAL4-VP16, which contains the strongest known activating region<sup>32</sup> (Fig. 4). The strength of E1a's activating region indicates that its activity is not the adventitious result of fusion to GAL4-(1–147).

### E1a contains a promoter-binding region

Like other activating regions, the VP16 activating region must be positioned in the vicinity of the promoter to function (reviewed in ref. 3). Thus, an important implication of the experiment shown in Fig. 3 is that GAL4-E1aΔN149 must contain an activity that directs the VP16 activating region to the wild-type E4 promoter. Neither the GAL4 binding domain, nor the VP16 activating region of GAL4-E1aΔN149-VP16, can provide this activity as GAL4-VP16 fails to activate the E4 promoter

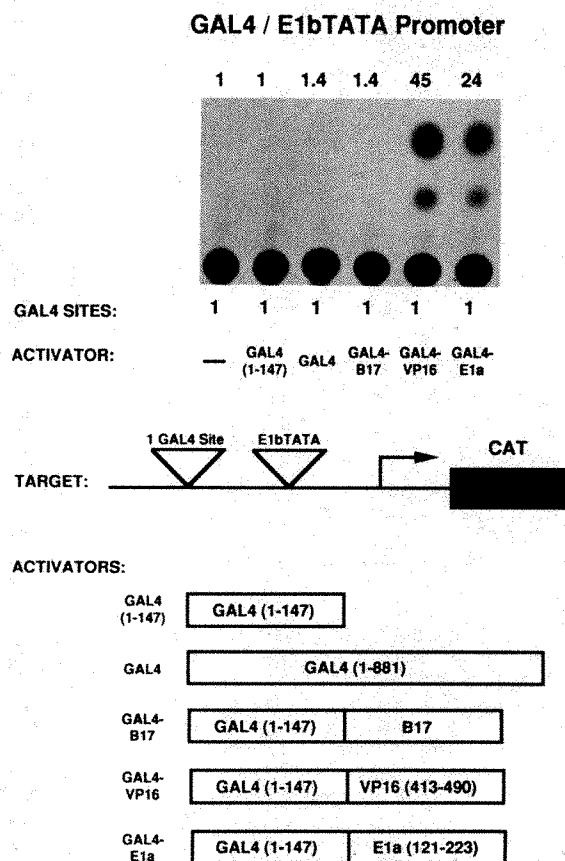


FIG. 4 E1a contains a powerful activating region. A reporter CAT plasmid, containing the E1bTATA promoter with a single GAL4 site inserted immediately upstream, was cotransfected with one of several plasmids that encode an activator. Relative CAT activity was quantitated and is indicated above each track. The E1bTATA promoter is activated detectably by both wild-type GAL4 and GAL4-B17 if an additional four GAL4 sites are inserted upstream, but not as efficiently as it is activated by GAL4-E1a (data not shown).

METHODS. Transfections were as described in the Fig. 1 legend. GAL4(1–147), GAL4-VP16 and GAL4-E1a are as described in Fig. 1. GAL4 is the wild-type GAL4 protein, amino acids 1–881, expressed from pSG4 (ref. 32). GAL4-B17 encodes an acidic *Escherichia coli* sequence fused to GAL4(1–147) and is expressed from pSGB17 (ref. 32).

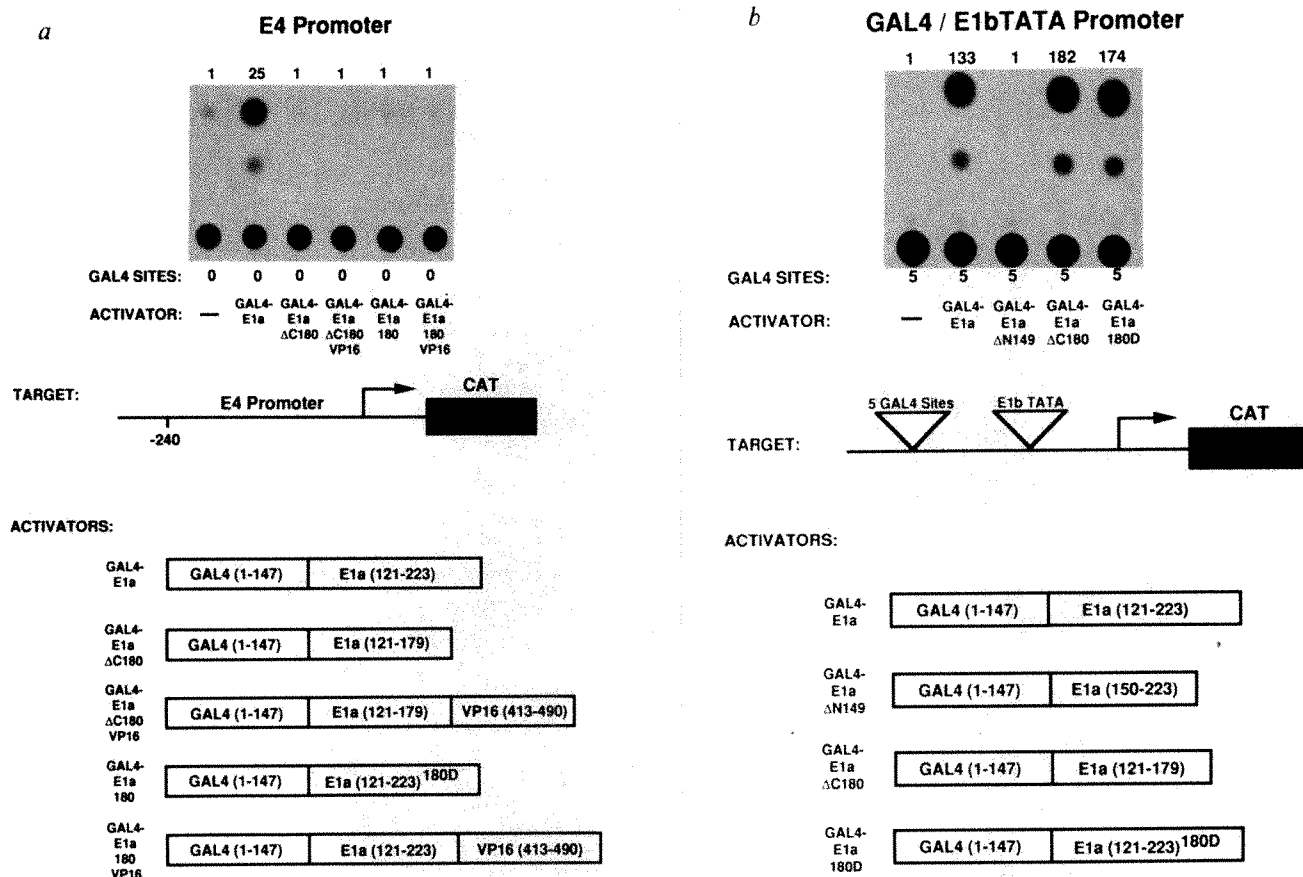


FIG. 5 The C terminus of E1a region 3 provides a promoter binding activity. A reporter CAT plasmid containing the E4 (a) or GAL4-E1bTATA (b) promoter was cotransfected with a plasmid encoding the activator indicated below each track. Relative CAT activity was quantitated and is indicated above each track.

METHODS. Transfections were as described in the Fig. 1 legend. Each activator is a derivative of GAL4-E1a described in the Fig. 1 legend. GAL4-E1aΔN149 is described in the Fig. 2 legend. GAL4-E1aΔC180 is E1a amino acids 121-179

fused to GAL4(1-147) and was created by a 3' *Bal*31 deletion of the *Clal*-*Xba*I fragment of the E1a 13S cDNA. GAL4-E1a180D contains a substitution at E1a amino acid 180 (Gly → Asp) and was constructed by replacing the *Clal*-*Xba*I fragment of GAL4-E1a with the *Clal*-*Xba*I fragment of the E1a activation mutant pH3G-13S-1098 (ref. 26). GAL4-E1aΔC180-VP16 and GAL4-E1a180D-VP16 contain the 78 C-terminal amino acids of the HSV1 protein fused in-frame to the C terminus of GAL4-E1aΔC180 and GAL4-E1a180D, respectively.

(Fig. 1a, GAL4-VP16). The only other sequences contained in GAL4-E1aΔN149 are E1a amino acids 150-223, suggesting that these E1a sequences provide a promoter-binding function. Below, we confirm this conclusion and further show that the sequences involved in promoter binding are required for E1a's natural activity.

Previous studies have shown that the C-terminal fragment of region 3 is important for wild-type E1a function<sup>24,26,28,30</sup>. The experiments in Fig. 5 indicate that the primary activity of this region is to bring the E1a activating region to the vicinity of its natural target promoters. Deletion of E1a amino acids 180-223, or substitution of amino acid 180, abolishes GAL4-E1a's ability to activate the E4 promoter, which lacks GAL4 sites (Fig. 5a, GAL4-E1aΔC180, GAL4-E1a180D; E4 promoter). The GAL4 DNA-binding domain restores the activity of these C-terminal mutants but only on a promoter that contains GAL4 sites (Fig. 5b, GAL4-E1aΔC180, GAL4-E1a180D; GAL4/E1bTATA promoter). Thus, the function of E1a amino acids 180-223 can be provided by the binding region of another activator.

The VP16 activating region, which restores the activity of region 3 N-terminal mutants, does not substitute for the C-terminal portion of region 3 (Fig. 5a, GAL4-E1aΔC180-VP16, GAL4-E1a180D-VP16). Conversely, the GAL4 DNA-binding domain, which restores the activity of region 3 C-terminal mutants, does not substitute for the N-terminal portion of region 3 (Fig. 5b, GAL4-E1aΔN149). Thus, the two ends of region 3 provide distinct activities, both of which are required for activation by wild-type E1a.

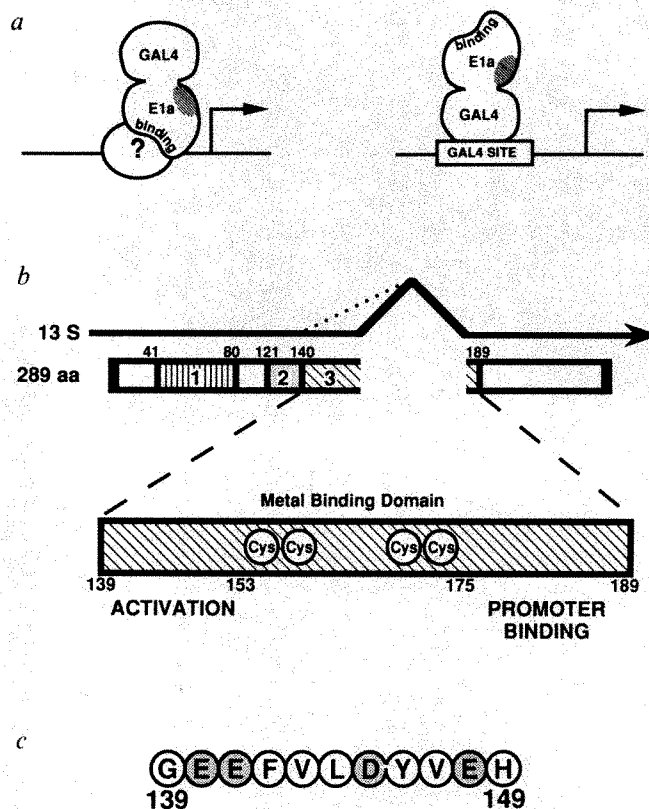
## Model

Taken together our results suggest that E1a functions naturally in the vicinity of the promoter. E1a contains both a promoter-binding region that directs E1a to its target promoters, and an activating region that functions when positioned at the promoter via the GAL4 DNA-binding domain or E1a's natural binding region (Fig. 6a). The activating and binding regions of cellular activators are modular, can be replaced by the corresponding region of an unrelated activator, and are functional independent of their position (reviewed in ref. 3). Similarly, we show that the N-terminal E1a activating region can be replaced by the VP16 activating region at the C-terminus, and the C-terminal E1a binding region can be replaced by the GAL4 DNA-binding domain at the N terminus. The activating regions of typical cellular activators are acidic (reviewed in ref. 3), and we note that the E1a activating region contains several acidic amino acids (Fig. 6c). The central portion of region 3 (amino acids 154-174), which contains a metal-binding domain<sup>46</sup>, is important for wild-type E1a activity<sup>30,46</sup>. Future experiments will determine if this domain is a component of the activating and/or the promoter-binding regions.

How is E1a targeted to the E4 and other viral early promoters? Our results indicate that E1a functions at the promoter, but there is no evidence that E1a binds to a specific DNA sequence (reviewed in ref. 17). It therefore seems probable that E1a's binding region recognizes a DNA-bound protein and not DNA. In this regard, E1a would resemble activating proteins such as



FIG. 6 Model of E1a action. *a*, Transcription activation by GAL4-E1a. A proposal for how GAL4-E1a interacts with target promoters that contain (right) or lack (left) GAL4-binding sites. GAL4-E1a protein is shown as a fusion of GAL4(1-147) and E1a. 'Binding' indicates the E1a promoter-binding region and shading indicates the E1a activating region. The unidentified component with which the E1a binding region interacts in the absence of GAL4 sites is shown by a question mark. *b*, Regions responsible for E1a activity. The structure of E1a 13S mRNA, which encodes the 289-amino acid protein, is shown on top. The intron removed by splicing is designated by a caret. The position of the 12S 5' splice site is indicated by a dotted line. The three conserved regions of the 289-amino acid E1a protein (box) are numbered 1, 2 and 3. The regions involved in activation are shown below. Four of the cysteines within the metal-binding region are shown. *c*, Amino-acid sequence shown to be involved in the E1a activating region. E1a amino acids 139 to 149 are shown. Negatively charged amino acids are stippled.



c-fos and HSV1 VP16, which interact with DNA-bound proteins and contribute additional activating regions<sup>4-10,45</sup>. One potential target protein is the cellular transcription factor ATF, which binds to multiple E1a-inducible adenoviral early promoters, including E4 (ref. 47).

If E1a functions at the promoter like a typical cellular activator, what accounts for its promiscuity? One possibility is that E1a's binding region recognizes more than one target protein. In fact, E1a is known to interact with multiple cellular proteins<sup>48,49</sup>. Perhaps E1a binds to some proteins, like ATF, avidly and to other cellular proteins with lower affinity. This could account for the variable effect of E1a on different pro-

motors<sup>17</sup>. In this regard, insertion of GAL4 sites greatly enhances activation of promoters that are otherwise weakly induced by GAL4-E1a, and modestly enhances activation of promoters that are otherwise efficiently induced by GAL4-E1a (Fig. 1; compare E4, MMTV and E1bTATA promoters). Alternatively, there may be additional mechanisms, unrelated to the one described here, by which E1a activates transcription.

In addition to adenovirus, other viruses such as pseudorabies virus<sup>50</sup>, bovine papilloma virus<sup>51</sup> and human T lymphotropic virus-1<sup>52</sup> encode proteins that activate transcription somewhat promiscuously. We speculate that these viral activators function in a similar way to E1a. □

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# Bars within bars: a mechanism for fuelling active galactic nuclei

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ACTIVE galactic nuclei (AGNs) are usually thought to be powered by accretion onto a supermassive black hole (SBH)<sup>1,2</sup>. Their luminosities, which may exceed  $10^{46}$  erg s<sup>-1</sup>, require mass accretion rates of  $\geq 1 M_{\odot}$  yr<sup>-1</sup> for reasonable mass-to-energy conversion efficiencies. Although this quantity of fuel could be supplied by the interstellar medium of the host galaxy, it is not obvious how it could be transported from typical galactic radii,  $\sim 10$  kpc, down to the scale of the SBH,  $\leq 10$  pc. We propose here a mechanism, applicable to AGNs and nuclear starburst galaxies, which brings in gas from large to small scales by successive dynamical instabilities. On the large scale, a stellar bar sweeps the interstellar medium into a gaseous disk of a few hundred parsecs in radius. Under certain conditions, this disk can become unstable again, allowing material to flow inwards until turbulent viscous processes control angular-momentum transport. This flow pattern may feed viscosity-driven accretion flows around a SBH, or lead to the formation of a SBH if none was present initially.

If the accretion flow is disk-like and is driven by local viscosity, then the radial inflow time can be expressed in terms of the standard  $\alpha$  parameter<sup>3</sup>, which is defined as a suitably averaged ratio of viscous stress to pressure inside the disk. For a disk with shear induced by a point-mass potential, the viscosity-controlled radial inflow time,  $t_{\text{visc}}$ , is

$$t_{\text{visc}} = \alpha^{-1} (v_{\phi}/c_s)^2 t_{\text{dyn}} \approx 1.2 \times 10^9 \alpha^{-1} v_{\phi 2} T_2^{-1} R_{10} \text{ yr} \quad (1)$$

where  $100 v_{\phi 2}$  km s<sup>-1</sup> is the orbital speed,  $10 R_{10}$  pc is the radius,  $t_{\text{dyn}}$  is the dynamical time  $R/v_{\phi}$ ,  $c_s$  is the sound speed in the disk, and  $100 T_2$  K is the gas temperature<sup>4,5</sup>. An alternative viscosity formulation, obtained by setting the coefficient of kinematic viscosity to  $\nu = \alpha c_s H$ , where  $H$  is the disk thickness, gives identical results for a non-self-gravitating disk, but leads to a slower inflow rate when vertical self-gravity is important. Because of the effects of self-gravity and fragmentation<sup>4-7</sup>, the disk feeding an AGN may resemble a system of gas clouds rather than a quasi-continuous fluid. In this case, the efficiency of angular-momentum transfer depends on the cloud-cloud collision timescale,  $t_{\text{coll}}$ , and the value of the effective  $\alpha$  is of order  $t_{\text{dyn}}/t_{\text{coll}}$ . We expect  $\alpha$  to be  $\leq 1$  at the onset of fragmentation, and to become smaller if the clouds contract further. If fragmentation is somehow suppressed, higher effective values of  $\alpha$  may be possible<sup>8</sup>, but a consideration of realistic heating and cooling processes suggests that this does not lead to a significant enhancement of inflow<sup>4,5</sup>. In regions where the stellar potential dominates over the potential of the SBH, the shear is reduced and the viscous inflow time is prolonged. We conclude, therefore that standard viscous processes are too slow to be effective in collecting gas liberated in the main body of the host galaxy. This can be overcome either if the gas is generated close to the SBH or if gas liberated at larger distances is brought in towards the SBH by a mechanism other than subsonic turbulent viscosity. For example, a large-scale non-axisymmetric disturbance of the galaxy, such as a stellar bar, is capable of inducing global shocks, thereby driving the inflow of gas towards the

centre. The amount of H I in spirals is observed to lie in the range  $10^8$ – $10^{10} M_{\odot}$ , and molecular gas may be more abundant by a factor of ten. Both the total H I, CO and H<sub>2</sub> content and the ratio of molecular gas to H I are maximal for spiral galaxies of type Sb to Sbc<sup>9,10</sup>. There is certainly enough gas present in spirals to power the whole range of AGN activity, provided that this gas can be swept inwards from several kpc down to tens of pc in a short time.

It has been known for some time that barred spiral galaxies are associated with Seyfert galaxies. Adams<sup>11</sup> noticed that a large number of Seyferts are barred. The 12 objects in his highest-resolution class are all barred spirals at some level, or show inner rings thought to be generated by bar instabilities<sup>12</sup>. Apart from ten obvious SB and SAB galaxies, this class contains NGC1068 and Mk10, which are observed to be barred<sup>13-15</sup>. In the next resolution class, Adams found that two thirds of the galaxies are SB or have rings. The remaining third either have an "amorphous body" or are seen edge-on, or their observation was subject to instrumental errors.

In a survey of a sample of Seyfert galaxies, Simkin *et al.*<sup>16</sup> concluded that there was an excess of bars, rings and oval distortions among Seyferts with respect to their control sample. A recent survey based on CCD images of a volume- and luminosity-limited sample by MacKenty<sup>17</sup> shows that Seyfert galaxies "nearly always possess mechanisms for transporting material into their nuclei (for example, peculiar, tidally interacting or barred galaxies)". In other respects the host galaxies appear normal, having colours, magnitudes and disk parameters typical of their morphological class. Clearly, the simplest interpretation of these observations is that all Seyferts are barred at some level.

The large-scale stellar bar cannot, however, be solely responsible for the AGN phenomenon. First, about half of the spiral galaxies in the sky are barred or show oval distortions whereas only a few per cent of them host AGNs (but see ref. 18). Second, the gas inflow generated by a barred rotating potential does not extend down to scales at which turbulent viscosity could take over. Numerical studies of the response of a compressible fluid to an imposed oval distortion of the potential indicate that, in these systems, the gaseous component loses angular momentum in a pair of shocks, and flows towards the centre of the galaxy in about ten rotation periods<sup>19,20</sup>. Given the fractional deviation of the potential from axial symmetry,  $\Delta\Phi$ , we can estimate the radius at which the shocks weaken and the inflow ceases to be dynamically driven. We assume that  $\Delta\Phi \approx \epsilon v_{\phi}^2$ , where  $\epsilon$  is of the order of 0.1. The condition for  $\Delta\Phi$  to cause a shock in the gas can be expressed as<sup>21</sup>  $c_g < 0.5(\gamma+1)\epsilon v_{\phi}$ , where  $c_g$  is the sound speed in the gas and  $\gamma$  is its adiabatic index. If the inner parts of the galaxy rotate as a solid body within a radius  $R_i$ , shocks will not be present within a radius  $R_d$  given by  $R_d = 2R_i/(\gamma+1)\epsilon M$ , where  $M$  is the Mach number ( $v/c_g$ ) of the flow at  $R_i$  and we have assumed  $c_g \ll v_{\phi}$ . Numerical simulations show that the gas can be swept up into a ring if inner Lindblad resonances (ILRs) are present, yielding a ring-like configuration of H II regions. If the bar distortion is strong, however, the positions of the ILRs cannot be calculated using the circular velocity curve<sup>22</sup>.

In summary, the gaseous inflow in a barred potential slows down at a radius  $R_d$ . We shall assume, for simplicity, that the net effect of a stellar bar on the gaseous component of the host galaxy is to sweep it into a disk with radial scale  $R_d \approx 0.1 R_b$ , where  $R_b$  is the radius of the bar. At  $R_d$ , viscous processes are still too slow to drive inflow. We propose instead that the gas which accumulates in the central kpc or so, as a consequence of the inflow in the stellar bar, forms a disk which, under certain conditions, becomes again dynamically unstable to form a gaseous bar. We derive a condition for this secondary instability in the context of a simple model, and argue that the resulting inflow can extend all the way into the centre of the galaxy, that is, to the inner  $\sim 10$  pc or so.

No single criterion is known for global stability of self-gravi-

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tating disks (gaseous or stellar) encompassing all possible disk models<sup>22,23</sup>. However, the stability of a large variety of disk models can be described by applying the semi-empirical criterion proposed by Ostriker and Peebles<sup>24</sup>, which is known to have a theoretical analogue for Maclaurin ellipsoids. A sufficient condition for instability is given by  $t = T_{\text{rot}}/|W| \geq t_{\text{crit}}$ , where  $T_{\text{rot}}$  is the kinetic energy of rotation of the disk,  $W$  is the gravitational energy of the system and  $t_{\text{crit}}$  is the maximum value of  $t$  required for stability. This criterion seems to apply to gaseous thin disks, which have  $t_{\text{crit}} \approx 0.14$  for secular instability and  $t_{\text{crit}} \approx 0.26$  for dynamical instability<sup>25,26</sup>. We model the system after the bar has brought the gas within the radius  $R_d$  in terms of three components: a cool gaseous disk having a Kuzmin density distribution with radial scale  $a$ ; a stellar disk having a similar density distribution but with a larger radial scale  $b$ ; and a Plummer-model halo, also with radial scale  $b$  (see, for example, ref. 23). Let the total mass of the system be  $M$ , a fraction  $g$  of which is in the gaseous disk and a fraction  $s$  in the stellar disk. The halo therefore contains a mass  $(1 - g - s)M$ . This particular choice of model is motivated by the fact that  $t$  can be evaluated analytically, but the conclusions drawn from it are expected to be at least qualitatively correct in a more general context.

We treat the potential of the stellar component as axisymmetric and fixed, and therefore include in the calculation of  $t$  only the kinetic energy of the gaseous disk:

$$T_{\text{rot}} = \frac{gf^2GM^2}{8a} \left[ g + \frac{4(1-g)}{(1+\beta)^2} \right] \quad (2)$$

where we have assumed that the disk rotates at every radius with a fraction  $f$  of the local circular speed for equilibrium ( $f=1$  indicates full rotational support), and have introduced  $\beta \equiv b/a$ , the ratio of length scales of the fixed stellar component to the gaseous component. From equation (2) and the expression for the potential energy,

$$|W| = \frac{GM^2}{4a} \left[ g^2 + \frac{4g(1-g)}{1+\beta} + \frac{s(2-2g-s)}{\beta} + \frac{3\pi}{8\beta} (1-s-g)^2 \right] \quad (3)$$

one can obtain an expression for  $t$  in terms of the mass fractions in the various components and the radial-scale ratio  $\beta$ . The physically interesting question is: given a certain gas fraction, by how much must the gas disk shrink radially in order to become unstable? A rough answer to this question can be obtained by setting  $t$  equal to  $t_{\text{crit}}$  and solving for  $\beta$ . In the limit  $g \ll 1$ , we find that the gaseous disk is unstable ( $t > t_{\text{crit}}$ ) if

$$\beta > \frac{2t_{\text{crit}}(1-h^2+3\pi h^2/8)}{g^2(f^2-2t_{\text{crit}})} \quad (4)$$

where  $h \equiv 1 - g - s$  is the halo mass fraction. The r.h.s. of eq. (4) is not very sensitive to the value of  $h$ , and equation (4) holds to better than 10% for  $g \leq 0.3$ , when compared with the exact evaluation of the Ostriker-Peebles criterion. Any effects of non-axisymmetry will probably enhance the instability.

Two aspects of equation (4) are worth mentioning: (1) for a given  $f$  and  $h$ ,  $\beta$  varies inversely with the gas fraction squared, and (2) the disk must be cold,  $f > \sqrt{2t_{\text{crit}}}$  for it ever to become unstable. This places stringent limits on the proposed mechanism. For example, in a cold disk with  $f=1$ ,  $h \approx 0.5$  and  $t_{\text{crit}} = 0.14$ ,  $\beta$  must be  $> 0.4/g^2$  for instability. If  $\beta=1$  initially and  $g \approx 0.2$ , then a reduction in size of the gas disk by a factor of ten would make it susceptible to global instabilities. It is important to note that the value of  $t_{\text{crit}} = 0.14$  used above corresponds to dynamical instability of stellar (collisionless) disks, but to only secular instability of gaseous disks. This is because gaseous disks have fewer degrees of freedom than stellar disks. Secular instability of a gaseous disk would occur on the viscous inflow time, and would therefore be too slow to be of interest. Dynamical instability of a smooth gaseous disk would require  $t_{\text{crit}} = 0.26$ , corresponding either to contraction by an additional factor of 2-3,

or to increase in  $g$  by a factor of about two. However, it is likely that the self-gravitating gaseous disk will be highly inhomogeneous, consisting of clouds which fill a rather small fraction of the volume<sup>5</sup>. If the collision frequency of clouds is small relative to the orbital frequency, the system will behave more like a stellar disk than a gaseous disk, and a lower value of  $t_{\text{crit}}$  will apply. Gravitational coupling of the gaseous disk to the stellar disk may also contribute to the instability<sup>5,27</sup>.

Therefore, we have established that a large-scale stellar bar sweeping the gas inward yields a disk, which can become unstable, if it is not too hot, and can feed a viscosity-driven flow. The unstable configuration is likely to resemble a bar-driven spiral<sup>25</sup>, which will transfer angular momentum outwards, leading to further contraction. At the same time, the velocity dispersion of inhomogeneities will tend to increase, but this energy will be dissipated through cloud-cloud collisions. To the extent that the system does not form stars, the inevitable consequence of sinking of the gaseous system towards the centre of the stellar system is that  $t$  will grow, and the gaseous system will become increasingly unstable. The degree to which the system forms stars before the gas reaches the centre must, in this model, determine whether the principal form of activity is a starburst or an AGN. The above argument implies that galaxies with a gas content of less than 10% will not display a bar within a bar and are therefore candidates for the generation of a starburst but not of a powerful AGN.

The process is not necessarily restricted to spirals. Elliptical galaxies are to some extent also supported by rotation. Material that is released at radii of a few kpc, and which is only 1% rotationally supported, would go into keplerian orbit at about one tenth of its original distance from the centre. Turbulent viscosity would still be insufficient to drive inflow, and the formation of a gaseous bar might occur even in this case.

Thus, a large-scale bar is a necessary but not sufficient condition for strong nuclear activity if the host is a spiral galaxy. Another stage of dynamical instability, which occurs in the gaseous disk that accumulates in the centre under the influence of the stellar bar, is required to feed an accretion disk at smaller scales, that is, such a process requires gaseous bars within stellar bars. A similar but tidally induced process was found in numerical simulations (L. Hernquist, preprint). Any process that suppresses this second stage of instability will prevent formation of the SBH and/or its subsequent fuelling, and is likely to lead to nuclear star formation or to low-level activity. This view is supported by recent evidence that most or perhaps all starbursts are barred<sup>28</sup>. The majority of spirals avoids becoming a conspicuous AGN or starburst. The most probable causes for this are relatively low gas content of the host, inefficiency of the stellar bar in sweeping all of the gas in, and the presence of ILRs.

The fact that activity seems to occur preferentially in intermediate morphological classes can be simply explained in terms of the relative abundance of gas-rich galaxies among those morphological types<sup>9,10</sup>. Additional factors, such as the length of the bar relative to the photometric galactic radius, may influence the correlation between activity and morphological type. It is plausible that nuclear activity in some galaxies may be suppressed at present because the SBH (or the nuclear starburst) has exhausted the available mass supply and must wait for new material to be brought in from large scales. The duration of the active phase of an AGN would be of the order of a few  $t_{\text{dyn}}$  at the bar radius, whereas the gas would be replenished only on the stellar-evolution timescale or on the timescale for capture of extragalactic clouds.

An important prediction of this model is that dynamical instabilities on scales of  $\sim 100$  pc must be present in the central regions of active galaxies, including ellipticals (such as powerful radio galaxies). Perhaps some evidence of the processes discussed here can be found in the Sérsic-Pastoriza galaxies, whose nuclei have been long recognized as unusually bright and disturbed<sup>29</sup>. All of these galaxies are barred at some level and some

of them are well-known today to be Seyferts and starbursts. More high-resolution optical and infrared observations of these objects and H I and CO observations of the innermost regions of moderately distant AGN are desirable.

We have not discussed the effects of environment on Seyferts and starbursts. The passage of a close companion may induce a stellar bar, but this may not be necessary for their production, as isolated galaxies may host bars which are relics from galaxy formation, or which have been generated spontaneously as a result of infall. □

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## A global assessment of natural sources of atmospheric trace metals

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A PROPER inventory of atmospheric emissions from natural sources is basic to our understanding of the atmospheric cycle of the trace metals (and metalloids), and is also needed for assessing the extent of regional and global pollution by toxic metals<sup>1</sup>. It is generally presumed that the principal natural sources of trace metals in the atmosphere are wind-borne soil particles, volcanoes, seasalt spray and wild forest fires<sup>2–6</sup>. Recent studies have shown, however, that particulate organic matter is the dominant component of atmospheric aerosols in non-urban areas<sup>7–10</sup> and that over 60% of the airborne trace metals in forested regions can be attributed to aerosols of biogenic origin<sup>11,12</sup>. Here I estimate that biogenic sources can account for 30–50% of the global baseline emissions of trace metals. For most of the toxic metals, the natural fluxes are small compared with emissions from industrial activities, implying that mankind has become the key agent in the global atmospheric cycle of trace metals and metalloids.

In compiling the emission factors used in the calculations (Table 1), the most recent data have been used; for older data, the lowest reported concentrations have been preferred. For

enrichment factors, a range of values<sup>1</sup>, rather than a single value, has been used. A range in the flux of material from each source has also been employed so as to assess the errors inherent in the reported emission intensities of trace metals.

The episodic nature of volcanic emissions makes it difficult to derive trace-metal emission rates using the enrichment-factor strategy<sup>12,13</sup>. There have been numerous studies, however, of the release of sulphur and trace metals from volcanoes and fumaroles, and a number of authors have estimated the outputs of trace metals by normalizing the metal release to the sulphur flux from this source<sup>15</sup>. I have adopted such an approach here, using a global volcanic sulphur flux of  $(15\text{--}50) \times 10^{12} \text{ g yr}^{-1}$  (refs 15, 16) and metal-to-sulphur ratios derived from published data<sup>13–17</sup>.

The release of large quantities of volatile non-methane hydrocarbons (NMHC) is well documented, particularly in forested areas<sup>18–20</sup>. For example, the average emission rates for NMHCs in the forested areas of the United States range from 450 to  $1,712 \mu\text{g m}^{-2} \text{ h}^{-1}$  (ref. 18). Biogenic emissions of isoprene and terpenes in the Amazon rain-forests have been estimated as 233 and  $1,040 \mu\text{g m}^{-2} \text{ h}^{-1}$  respectively<sup>19</sup>. Rasmussen and Khalil<sup>20</sup> have calculated the global atmospheric flux of isoprene to be  $450 \times 10^{12} \text{ g yr}^{-1}$ , and the combined releases of isoprene and terpenes have been suggested<sup>19</sup> to be equivalent to ~0.7% of the net global primary production. Thus the NMHC flux of  $100\text{--}500 \mu\text{g m}^{-2} \text{ h}^{-1}$  used here is clearly a conservative figure. Terpenes and isoprene, the two dominant components of NMHCs, are known to form strong complexes with many trace metals<sup>21</sup>, and thus should play a part in the transfer of metals to the atmosphere. As far as I know, however, the NMHCs have not been analysed for trace metals, so that the emission factors listed in Table 1 are subject to a large uncertainty. They are based on reported flux measurements and the concentrations of methylated compounds of Hg, As, Pb and Se in the atmosphere<sup>6,14,24</sup>. For the other elements, the emission factors are based on metal concentrations in the surface organic microlayers of aquatic ecosystems<sup>22,23</sup>.

The average concentration of particulate organic carbon (POC; derived from organic matter which is the dominant component of atmospheric aerosols) in the Amazon rain-forests has been reported<sup>9</sup> to be  $8.9 \mu\text{g C m}^{-3}$  in the mixed layer and  $2.6 \mu\text{g C m}^{-3}$  in the free troposphere. These reported POC concentrations in forest ecosystems are considerably higher than the mean value of  $0.06 \mu\text{g C m}^{-3}$  in the marine atmosphere of the Southern Hemisphere<sup>44</sup>. The carbon isotope composition suggests, however, that most of the marine POC is also derived from natural land-based sources<sup>44</sup>. The annual flux of particulate organic matter (POM) to the atmosphere,  $Q$ , can be estimated from

$$Q = V \times B \times 2.2 \times (3\text{--}6) \times 10^{13}$$

where  $V$ , the total deposition velocity of the aerosols, is estimated to be  $1.0 \text{ cm s}^{-1}$  (ref. 44), and  $b$ , the average POC concentration, is  $3\text{--}9 \mu\text{g m}^{-3}$ . The figure of 2.2 is used to relate organic carbon to organic matter<sup>47</sup>, and the forested area is believed to be  $(3\text{--}6) \times 10^{13} \text{ m}^2$  (ref. 51). These values yield a global atmospheric POM flux of about  $(1\text{--}5) \times 10^{14} \text{ g yr}^{-1}$ . The POM flux used here is thus higher than the value of  $27 \times 10^{12} \text{ g yr}^{-1}$  reported recently by Cachier *et al.*<sup>44</sup>. The global flux of material from the other non-biogenic sources are summarized in Table 1.

There is a wide range in the estimated total emission of each metal from any given source (Table 1), reflecting the large dispersion in the emission factors used and in the published estimates of the mass flux from individual sources. There are significant gaps in the data, demonstrating the need for further study of the role of biological processes in the emission of trace metals. The proposed limits on the fluxes of metals from each source serve to indicate that current data are sufficient for only order-of-magnitude estimates of the global emission intensities. In view of this, the central (or median) values of the ranges

TABLE 1 Worldwide emissions of trace metals from natural sources ( $\times 10^9$  g yr $^{-1}$ )

	As	Cd	Co	Cr	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Se	V	Zn
Wind-borne soil particles (0.6–5) $\times 10^{14}$ g														
Emission factor ( $\mu\text{g g}^{-1}$ )†	5–10	0.2–0.8	10–15	60–100	15–30	0.05–0.2	700–800	2.0–5.0	30–40	5.0–15	1.0–3.0	0.1–0.7	20–60	50–80
Amount emitted: Range	0.3–5.0	0.01–0.4	0.6–7.5	3.6–50	0.9–15	0–0.1	42–400	0.12–2.5	1.8–20	0.3–7.5	0.06–1.5	0.01–0.35	1.2–30	3.0–35
Median value	2.6	0.21	4.1	27	8.0	0.05	221	1.3	11	3.9	0.78	0.18	16	19
Seasalt spray§ (0.1–1.0) $\times 10^{16}$ g														
Emission factor ( $\mu\text{g g}^{-1}$ )	186–314	2.8–11	1.4–14	30–140	230–690	0.14–4.3	20–170	8.6–4.3	93–260	115–285	28–114	43–114	143–714	17–86
Amount emitted: Range	0.19–3.1	0–0.11	0–0.14	0.03–1.4	0.23–6.9	0–0.04	0.02–1.7	0.01–0.43	0.01–2.6	0.02–2.8	0–1.1	0–1.1	0.14–7.2	0.02–0.86
Median value	1.7	0.06	0.07	0.07	3.6	0.02	0.86	0.22	1.3	1.4	0.56	0.55	3.1	0.44
Volcanoes¶ (15–50) $\times 10^{12}$ g S														
Metal/S ratio ( $\times 10^{-4}$ )	0.1–1.5	0.09–0.3	0.01–2.5	0.5–5.8	0.6–3.5	0.02–0.4	2.8–16	0.03–0.15	0.62–5.6	0.36–1.2	0–0.27	0.07–0.35	0.14–2.1	0.21–3.8
Amount emitted: Range	0.15–7.5	0.14–1.5	0.02–1.9	0.81–29	0.9–18	0.03–2.0	4.2–80	0.04–0.75	0.93–28	0.54–6.0	0.01–1.4	0.10–1.8	0.21–11	0.31–19
Median value	3.8	0.82	0.96	15	9.4	1.0	42	0.4	14	3.3	0.71	0.95	5.6	9.6
Wild forest fires** (2–15) $\times 10^{14}$ g														
Emission factor ( $\mu\text{g g}^{-1}$ )††	0–0.25	0–0.15	0.1–0.4	0–0.12	0.5–50	0–0.3	6.0–30	0.2–0.75	0.5–3.5	0.1–2.5	0–0.3	0–0.35	0.1–2.4	1.5–10
Amount emitted: Range	0–0.38	0–0.22	0.02–0.6	0–0.18	0.1–7.5	0–0.05	1.2–45	0.04–1.1	0.1–4.5	0.06–3.8	0–0.45	0–0.52	0.02–3.6	0.3–15
Median value	0.19	0.11	0.31	0.09	3.8	0.02	23	0.57	2.3	1.9	0.22	0.26	1.8	7.6
Biogenic														
Continental particulates‡‡ (1–5) $\times 10^{14}$ g														
Emission factor ( $\mu\text{g g}^{-1}$ )§§	0.2–1.0	0–0.6	0.5–2.0	1.0–4.0	1.0–10	0–0.8	20–100	0.4–1.5	1.0–6.0	0.2–5.0	0–0.6	0–0.5	0.2–3.5	3–10
Amount emitted: Range	0.2–0.5	0–0.83	0.05–1.0	0.1–2.0	0.1–5.0	0–0.04	4–50	0.04–0.75	0.1–1.0	0.02–2.5	0–0.4	0–0.25	0.04–1.8	0.3–5.0
Median value	0.26	0.15	0.52	1.0	2.6	0.02	27	0.40	0.51	1.3	0.2	1.12	0.92	2.6
Continental volatiles    (2–25) $\times 10^{13}$ g														
Emission factor ( $\mu\text{g g}^{-1}$ )¶¶	1.0–10	0–0.3	0–0.5	0–0.4	0.3–2.5	0.5–5.0	1.0–10	0–0.5	0.1–0.8	0.2–1.5	0–0.3	5–20	0.3–1.0	0.5–20
Amount emitted: Range	0.03–2.5	0–0.8	0–0.12	0–0.10	0.01–0.62	0.02–1.2	0.03–2.5	0–0.12	0–0.20	0.01–0.38	0–0.8	0.15–5.0	0.01–0.25	0.02–5.0
Median value	1.3	0.04	0.06	0.05	0.32	0.61	1.3	0.06	0.10	0.20	0.04	2.6	0.13	2.5
Marine### (8–30) $\times 10^{13}$ g														
Emission factor ( $\mu\text{g g}^{-1}$ )¶¶¶	2.0–15	0–0.3	0–0.5	0–0.4	0.3–2.5	0.5–5.0	1.0–10	0–0.5	0.1–0.8	0.2–1.5	0–0.3	5–30	0.3–1.0	0.5–20
Amount emitted: Range	0.16–4.5	0–0.1	0–0.15	0–0.12	0.02–0.75	0.04–1.5	0.08–3.0	0–0.15	0.01–0.45	0.02–0.45	0–0.1	0.4–9.0	0.02–0.3	0.04–6.0
Median value	2.3	0.05	0.08	0.06	0.39	0.77	1.5	0.08	0.12	0.24	0.05	4.7	0.16	3.0
Total emission: Range	0.86–23	0.15–2.6	0.69–11	4.5–83	2.3–54	0.1–4.9	52–582	0.14–5.8	3.0–57	0.97–23	0.07–4.7	0.66–18	1.6–54	4.0–86
Median value	12	1.3	6.1	44	28	2.5	317	3.0	30	12	2.4	9.3	28	45

The amount of each metal emitted is derived by multiplying its emission factor by the flux of material from the particular source.

† The range in mass of wind-borne soil particles was reported by Prospero *et al.*<sup>32</sup>

‡ The ranges in average trace-metal concentration of the soil are based on the compilations and reviews by Bowen<sup>33</sup> and Ure and Berrow<sup>34</sup>.

§ Based on estimates by Blanchard<sup>35</sup>.

|| Based on the recently reported concentrations of trace metals in sea water<sup>14,36–41</sup>, an average seawater salinity of 35‰, and an enrichment factor for sea salt of 10 for Mn, Mo, Cr and V, 50 for Se, Sb, Hg, Co, Ni, Cd and Pb, and 200 for Cu, Zn, Pb and Cd<sup>28,42,43</sup>.

¶ From Berresheim and Jaeschke<sup>16</sup> and Lambert *et al.*<sup>15</sup>.

¶¶ Based on trace-metal concentrations in volcanic emanations reported in refs 5, 13, 15–17 and 44.

\*\* Quantity of forest biomass consumed by wild fires derived from the data given in refs 45–47.

†† Derived from the trace-metal concentrations in land plants<sup>4,13</sup> and assuming burning yields of 30% for Mn and Cr, 50% for Pb, Co, Cd, Mo, Sb, Cu and Zn, and 70% for Se, Hg, V and As (refs 46, 49).

‡‡ See text.

§§ Trace-metal concentrations in land plants, which are used to derive the emission factors in††.

||| The average emission of volatile non-methane natural hydrocarbons (NMHC) in the United States has been estimated to be 450, 884 and 1,712  $\mu\text{g m}^{-2} \text{h}^{-1}$ , respectively (refs 25–27). My calculation is based on NMHC flux of only (100–500)  $\mu\text{g m}^{-2} \text{h}^{-1}$  from (3–6)  $\times 10^{13} \text{ m}^2$  of forested area<sup>45,50</sup>.

¶¶ The data listed for As, Hg and Se are based on published measurements of volatilization rates (for review see, for example, refs 3, 14, 22, 23, 30, 31). The emission factors reported for the other metals are based on their concentrations in surface organic microlayers.

### I have assumed that the flux of volatile NMHCs from the 170  $\times 10^{12} \text{ m}^2$  of coastal shelf and upwelling areas of the ocean<sup>51</sup> is 50–200  $\mu\text{g m}^{-2} \text{h}^{-1}$ .

given should be used in preference to the mean values, used by other authors.

Based on these data, my inventories suggest that biogenic sources can account, on average, for over 50% of the Se, Hg and Mo, and 30–50% of the As, Cd, Cu, Mn, Pb and Zn, released annually to the atmosphere from natural sources (Table 1). This important source of metals in the atmosphere has been either ignored or badly underestimated in most of the previously published inventories of trace-metal emissions from natural sources (for example, refs 2–6). This large estimated biogenic flux is consistent with recent reports<sup>10,11</sup> that the vegetation accounts for up to 90% of the airborne trace metals in the Amazon Basin. Biologically mediated volatilization processes can account for 30–50% of the total Hg, As and Se emitted annually, whereas the other metals are primarily associated with pollens, spores, waxes, leaf and needle fragments, fungi, algae and so on.

Records in polar ice layers<sup>26</sup> indicate that major volcanic eruptions often result in massive outputs which dominate the global atmospheric cycle of trace metals. My global inventory suggests that volcanic emanations can account for 40–50% of the Cd and Hg and 20–40% of the As, Cr, Cu, Ni, Pb and Sb emitted annually (Table 1). The data for volcanogenic sources (Table 1) are consistent with those reported recently by Lambert *et al.*<sup>15</sup>, who used the release of <sup>210</sup>Pb to normalize the SO<sub>2</sub> and trace-metal outputs. Patterson and Settle<sup>5</sup>, however, estimated the annual volcanic flux of Pb to be only 0.5  $\times 10^{12}$  g (corrected from the value of 1.2  $\times 10^{12}$  g published by these authors), a

value which is lower than the median value but which falls within the range shown in Table 1. The sulphur flux of (15–50)  $\times 10^{12}$  g used here presumably includes sulphur (and hence is accompanied by trace metals) from both eruptive and non-eruptive activity; fumarolic sulphur emission alone has been estimated to be (15–27)  $\times 10^{12}$  g yr $^{-1}$  (ref. 27).

With the exception of As (14%) and Sb (16%), seasalt aerosols seem to account for <10% of atmospheric trace metals from natural sources. The seasalt emission rates reported by Wiesel *et al.*<sup>28</sup> generally fall towards the upper end of the ranges in Table 1. At present, it is impossible to differentiate between biogenic and (seasalt) spray origins of trace metals in the marine atmosphere, although my inventory tends to favour biological processes as the main contributor. Soil-derived dusts can account for over 50% of the total Cr, Mn and V emissions, as expected. This source may also account for 20–30% of the Cu, Mo, Ni, Pb, Sb and Zn, but <10% of the Hg and Se released annually to the atmosphere. By ignoring biogenic outputs, previous estimates have generally overestimated the importance of resuspended soil particles in the atmospheric trace-metal budget.

The data in Table 1 pertain to emissions in recent years and therefore include some recycled anthropogenic metals. For some metals, the emission rates in prehistoric times were conceivably lower than those calculated today. Biological processing of trace metals dispersed in the environment by human activities is well documented (see, for example, refs 14, 24, 31, 33, 39). It is impossible, however, to use the available information to quantify the contribution of recycled material to the total flux of any of



TABLE 2 Natural versus anthropogenic emissions of trace metals to the atmosphere in 1983

Trace metal	Anthropogenic source	Natural source	Total emission	Natural/total emissions*
As	19 (12–26)	12 (0.86–23)	31 (13–49)	0.39
Cd	7.6 (3.1–12)	1.3 (0.15–2.6)	8.9 (3.2–15)	0.15
Cr	30 (7.3–54)	44 (4.5–83)	74 (12–137)	0.59
Cu	35 (20–51)	28 (2.3–54)	63 (22–105)	0.44
Hg	3.6 (0.91–6.2)	2.5 (0.10–4.9)	6.1 (1.0–11)	0.41
Mn	38 (11–66)	317 (52–582)	355 (63–648)	0.89
Mo	3.3 (0.79–5.4)	3.0 (0.14–5.8)	6.3 (0.93–11)	0.48
Ni	56 (24–87)	30 (3.0–57)	86 (27–144)	0.35
Pb	332 (289–376)	12 (0.97–23)	344 (290–399)	0.04
Sb	3.5 (1.5–5.5)	2.4 (0.07–4.7)	5.9 (1.6–10)	0.41
Se	6.3 (3.0–9.7)	9.3 (0.66–18)	16 (2.5–24)	0.58
V	86 (30–142)	28 (1.6–54)	114 (32–220)	0.25
Zn	132 (70–194)	45 (4.0–86)	177 (74–280)	0.34

All figures in units of  $10^9 \text{ g yr}^{-1}$ . Emissions from anthropogenic sources are from Nriagu and Pacyna<sup>1</sup>; these are median values, with the ranges in estimated emissions given in parentheses.

\* Median values only.

the trace metals and metalloids.

The total outputs listed in Table 1 do not agree with the inventories of natural source emissions published by other authors<sup>2,4,6</sup>. Because of refinement of the biogenic component, the data given here are generally higher than those given in my previous report<sup>2,9</sup>. In spite of discrepancies in source intensities, the calculated As flux is in good agreement with the value of  $7.8 \times 10^9 \text{ g yr}^{-1}$  reported by Walsh *et al.*<sup>2</sup>, but is less than half of that reported more recently by Chivers and Peterson<sup>30</sup>. The total annual Hg flux of  $(4\text{--}5) \times 10^9 \text{ g}$  reported by Fitzgerald<sup>31</sup> and the Se flux of  $(6\text{--}13) \times 10^9 \text{ g}$  reported by Mosher and Duce<sup>14</sup> are in good agreement with the inventories for these two elements in Table 1.

A comparison of the median values of the worldwide emissions of trace metals from natural and anthropogenic sources suggests that industrial emissions of Pb, Cd and Zn exceed the flux from natural sources by factors of 18, 5 and 3 respectively (see Table 2). Industrial discharges are apparently exercising a profound influence on the atmospheric cycles of these three toxic metals. For As, Hg, Ni, Sb and V, the contributions from anthropogenic sources may exceed those from natural sources by 100–200%. Thus it seems that mankind has become the key agent in the global atmospheric cycle of the toxic metals and metalloids. □

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## Studies of static magnetic order in electron-superconductors and their parent compounds

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UNTIL recently, all known high- $T_c$  superconductors involved conduction by holes; the doping of holes into the parent compound, for example by the substitution of  $\text{Sr}^{2+}$  or  $\text{Ba}^{2+}$  for  $\text{La}^{3+}$  in  $\text{La}_2\text{CuO}_{4-y}$ , results in a metallic state whose electronic ground state is superconducting. Recently, Tokura *et al.*<sup>1</sup> have discovered a series of superconducting copper oxides of general formula  $\text{Ln}_{2-x}\text{Ce}_x\text{CuO}_{4-y}$  (where Ln represents Pr, Nd, Sm), with transition temperatures as high as  $T_c \approx 24 \text{ K}$ , in which the charge carriers are electrons. These compounds have the  $\text{Nd}_2\text{CuO}_{4-y}$  structure, which is similar to that of  $\text{La}_2\text{CuO}_{4-y}$  except for a different arrangement of the oxygen atoms that results in the absence of Cu–O octahedra. Superconductivity is achieved by doping  $\text{Ce}^{4+}$  for  $\text{Ln}^{3+}$  and making the samples oxygen-deficient. Here we show, using muon spin rotation, that the parent compounds  $\text{Ln}_2\text{CuO}_{4-y}$  of these electron-superconductors exhibit static magnetic order below 300 K, which is similar to that observed in the parent compounds of hole-superconductors. In addition, fairly random static magnetic order has been observed in the non-superconducting material  $\text{Nd}_{1.90}\text{Ce}_{0.10}\text{CuO}_{4-y}$  below about 240 K, but no detectable static magnetic order was observed above 4 K in a superconducting sample  $\text{Nd}_{1.8}\text{Ce}_{0.16}\text{CuO}_{4-y}$  (with a  $T_c$  of 24 K). Hence, electron

doping seems not to fully destroy the magnetic order until the material becomes superconducting. The observation of static magnetic order in these new systems demonstrates that the electronic properties of these copper oxide planar systems are at least partially symmetric with respect to electron and hole doping.

The technique of positive muon spin rotation<sup>2</sup> ( $\mu$ SR) has been applied extensively to the study of high- $T_c$  superconductors and related materials<sup>3-8</sup>. In solids, the positive muon serves as a sensitive probe of the internal magnetic fields at interstitial sites(s), where the muon resides. In an antiferromagnet one observes one or more discrete oscillation frequencies in zero applied field<sup>9-11</sup>, which are independent of the relative orientation of the local field with respect to the initial polarization and are thus the same for a powder and a crystal. In a disordered magnetic system such as a spin glass, in which there is a broad distribution of local-field magnitudes at the muon site(s), the coherence of the muon spins is quickly lost, and this appears as a rapid depolarization of the muon polarization<sup>12</sup>.

The application of a weak external transverse magnetic field allows one to determine the volume fraction of the sample that

is magnetically ordered. Muons that reside in sites at which there is an appreciable internal magnetic field ( $\sim 30$  G or greater) are quickly depolarized, whereas those in sites with no local ordered field precess in the applied external field<sup>11</sup>. Thus the fraction of the muon polarization that precesses coherently corresponds to the volume fraction of the sample that has no appreciable static moment.

The pure parent compounds were prepared at DuPont Experimental Station by solid-state reactions of the rare-earth oxides ( $\text{Nd}_2\text{O}_3$ ,  $\text{Pr}_2\text{O}_3$ ,  $\text{Sm}_2\text{O}_3$ ) with  $\text{CuO}$  at  $1,000$ – $1,100^\circ\text{C}$ , as described elsewhere (J. Gopalkrishnan *et al.*, preprint). X-ray diffraction indicated that the samples were substantially single-phase; powder neutron diffraction revealed the crystal structure to be that of  $\text{Nd}_2\text{CuO}_4$  (described in ref. 1).

The experiments were performed at the M20 surface muon channel at TRIUMF which provides a beam of  $\sim 100\%$  spin-polarized positive muons. Figure 1 shows the zero-field muon polarization at low temperature in  $\text{Nd}_2\text{CuO}_{4-y}$ ,  $\text{Pr}_2\text{CuO}_{4-y}$  and  $\text{Sm}_2\text{CuO}_{4-y}$ . Over much of the temperature range the coherence of the muon spins is lost after a few periods of oscillation. This

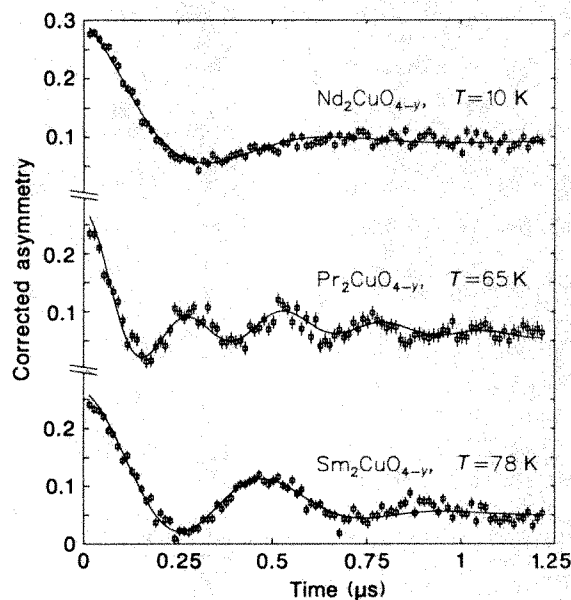


FIG. 1 Zero-field  $\mu$ SR spectra for  $\text{Nd}_2\text{CuO}_{4-y}$ ,  $\text{Pr}_2\text{CuO}_{4-y}$  and  $\text{Sm}_2\text{CuO}_{4-y}$  below the ordering temperature.

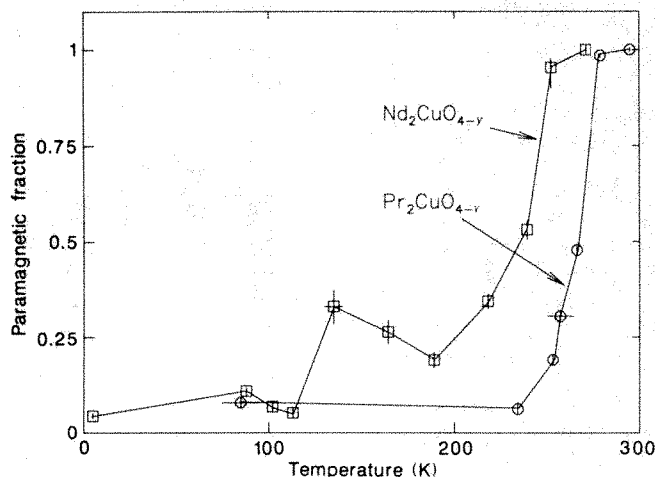


FIG. 2 Temperature dependence of the disordered (paramagnetic) volume fraction in  $\text{Nd}_2\text{CuO}_{4-y}$  and  $\text{Pr}_2\text{CuO}_{4-y}$ .

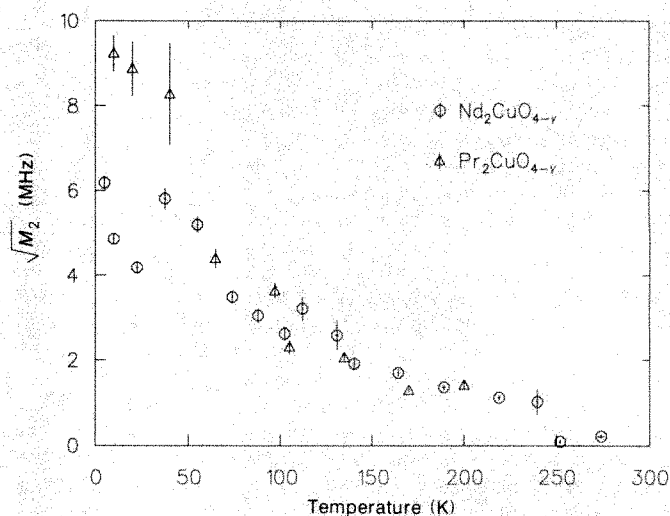


FIG. 3 Temperature dependence of the square root of the muon spin relaxation second moment, measured in zero magnetic field for  $\text{Nd}_2\text{CuO}_{4-y}$  and  $\text{Pr}_2\text{CuO}_{4-y}$ .

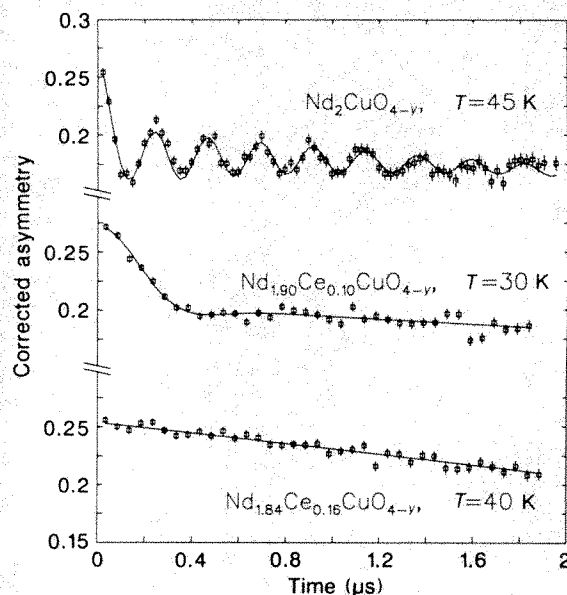


FIG. 4 Zero-field  $\mu$ SR spectra for reduced samples of non-superconducting  $\text{Nd}_2\text{CuO}_{4-y}$  and  $\text{Nd}_{1.90}\text{Ce}_{0.10}\text{CuO}_{4-y}$ , and superconducting  $\text{Nd}_{1.84}\text{Ce}_{0.16}\text{CuO}_{4-y}$ .

indicates that the width of the local distribution at the muon site(s) is of the same order of magnitude as the average field. At some temperatures, however, longer-lived precession is clearly visible, indicating that at least short-range magnetic order (rather than mere spin freezing) does occur in these materials. Figure 2 shows the paramagnetic or non-ordered sample volume fraction as a function of temperature for  $\text{Nd}_2\text{CuO}_{4-y}$  and  $\text{Pr}_2\text{CuO}_{4-y}$ .

We have parameterized the muon relaxation function as  $\exp(-\frac{1}{2}\sigma^2 t^2) \cos(\omega t)$ , corresponding to a gaussian distribution of local fields centred about  $\omega/\gamma_\mu$ . The fitted frequency  $\omega$  and the relaxation rate  $\sigma$  are highly correlated when there is such rapid relaxation, so that we then extract the initial second moment of the spin relaxation, given by  $M_2 = \sigma^2 + \omega^2$ . The square root of the second moment is plotted as a function of temperature in Fig. 3 for  $\text{Nd}_2\text{CuO}_{4-y}$  and  $\text{Pr}_2\text{CuO}_{4-y}$ . The low-temperature values of  $M_2$  are consistent with values reported for  $\text{La}_2\text{CuO}_{4-y}$  (refs 9, 10). The similarity in size of the ordered moment leads us to conclude that it is the copper-atom spins that are ordered (as in  $\text{La}_2\text{CuO}_{4-y}$ ), because if the rare-earth atoms were ordered, the ordered moment would be much larger. In a neutron scattering experiment on an essentially similar sample of  $\text{Pr}_2\text{CuO}_{4-y}$  below  $T = 300$  K, Cox *et al.* (personal communication) have observed Bragg scattering that is consistent with antiferromagnetic ordering of copper spins, and an earlier electron paramagnetic resonance study<sup>13</sup> found indications of antiferromagnetic order of copper spins in the  $\text{Ln}_2\text{CuO}_{4-y}$  materials.

The temperature at which the spins freeze (that is, at which a large static component of the local moment develops) is  $\sim 300$  K for all three compounds. In each of these materials, however, the degree of randomness in the magnetic order exhibits a complex temperature dependence, which will be discussed fully elsewhere.

To study the effects of carrier doping on the magnetic behaviour of these materials, we studied three specimens in the series  $\text{Nd}_{2-x}\text{Ce}_x\text{CuO}_{4-y}$ : the undoped parent compound  $\text{Nd}_2\text{CuO}_{4-y}$ , a partially doped, non-superconducting sample  $\text{Nd}_{1.90}\text{Ce}_{0.10}\text{CuO}_{4-y}$  and a more fully doped superconducting sample  $\text{Nd}_{1.84}\text{Ce}_{0.16}\text{CuO}_{4-y}$ , for which  $T_c = 24$  K. All three samples, which were prepared at the University of Tokyo<sup>1</sup>, were reduced at  $900^\circ\text{C}$  under an  $\text{Ar}/\text{O}_2$  atmosphere with a low oxygen partial pressure, making them oxygen-deficient ( $y > 0$ ). Representative  $\mu\text{SR}$  spectra in zero applied field are shown in Fig. 4.

In both non-superconducting samples, static internal fields were observed, indicating some sort of magnetic order or spin freezing. The presence of coherent oscillations (Fig. 4) indicates that more uniform magnetic order occurs in the oxygen-deficient  $\text{Nd}_2\text{CuO}_{4-y}$  than in the more fully oxygenated sample shown in Fig. 1. The temperature dependence of this uniformity of order is, however, also complex in this sample. The onset temperature of spin-freezing in the  $x = 0.10$  Ce-doped sample was reduced to  $T_N \approx 240$  K, and the complete sample volume was ordered or frozen by  $T = 150$  K. The magnitude of the static moment at low temperature was approximately the same as that of the parent compound  $\text{Nd}_2\text{CuO}_{4-y}$ . The presence of static magnetic order at such a high temperature in a material with substantial doping contrasts with the  $\text{La}_{2-x}\text{Sr}_x\text{CuO}_{4-y}$  system: magnetic order is lost above about 5 K in  $\text{La}_{1.92}\text{Sr}_{0.08}\text{CuO}_{4-y}$ , and at lower temperatures for higher Sr concentrations<sup>14</sup>.

The superconducting  $\text{Nd}_{1.84}\text{Ce}_{0.16}\text{CuO}_{4-y}$  sample exhibited no detectable static magnetic order above 4 K. The zero-field relaxation increased somewhat below 60 K (Fig. 4) but was still much less than that seen in non-superconducting samples.

In all of the non-superconducting specimens studied, we observe a more random and complicated magnetic behaviour as a function of temperature than the simple antiferromagnetic ordering observed in the  $\text{La}_2\text{CuO}_{4-y}$  system<sup>9,10</sup>. This behaviour may be due to changes in the ordered spin structure with

temperature, resulting in changing magnetic environments for the muon site(s). An orthorhombic distortion in the  $\text{La}_2\text{CuO}_{4-y}$  system stabilizes the magnetic structure of the body-centred copper atoms. In the Ce-doped materials, however, the structure remains tetragonal over the full temperature range and thus the body-centred copper atoms do not have a specific energetically preferred orientation. As there are two equivalent possible orientations of the copper-atom moments, a large degree of randomness in the magnetic order might be expected. Neutron-scattering measurements on single-crystal specimens are necessary to resolve this point, because  $\mu\text{SR}$  by itself cannot easily determine the ordered spin structure or the range of the order, as the muons are affected mainly by the nuclei of their nearest neighbours.

Thus the magnetic behaviour of the parent compounds of the electron-superconductors is rather similar to that of the corresponding hole-superconductors, in that ordering occurs at roughly the same temperature with an equivalent static moment. In view of the proposed link between magnetic and superconducting properties in these materials, such information may be of great importance in determining how, if at all, the mechanisms for superconductivity differ for the two classes of compound.  $\square$

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## Wavelet analysis of turbulence reveals the multifractal nature of the Richardson cascade

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THE central problem of fully developed turbulence is the energy-cascading process. It has resisted all attempts at a full physical understanding or mathematical formulation. The main reasons for this failure are related to the large hierarchy of scales involved, the highly nonlinear character inherent in the Navier–Stokes equations, and the spatial intermittency of the dynamically active regions. Richardson<sup>1</sup> has described the interplay between large and small scales with the words ‘Big whirls have little whirls which feed on their velocity ... and so on to viscosity’, and the phenomenon so described is known as the Richardson cascade. This local interplay also forms the basis of a theory by Kolmogorov<sup>2</sup>. It was later realized that the cascade ought to be intermittent, and Mandelbrot<sup>3</sup> has given a fractal description of the intermittency of the fine structure. A particular case that emphasizes the dynamical aspect of the fractal models is the  $\beta$ -model<sup>4</sup>, in which the flux of energy is transferred to only a fixed



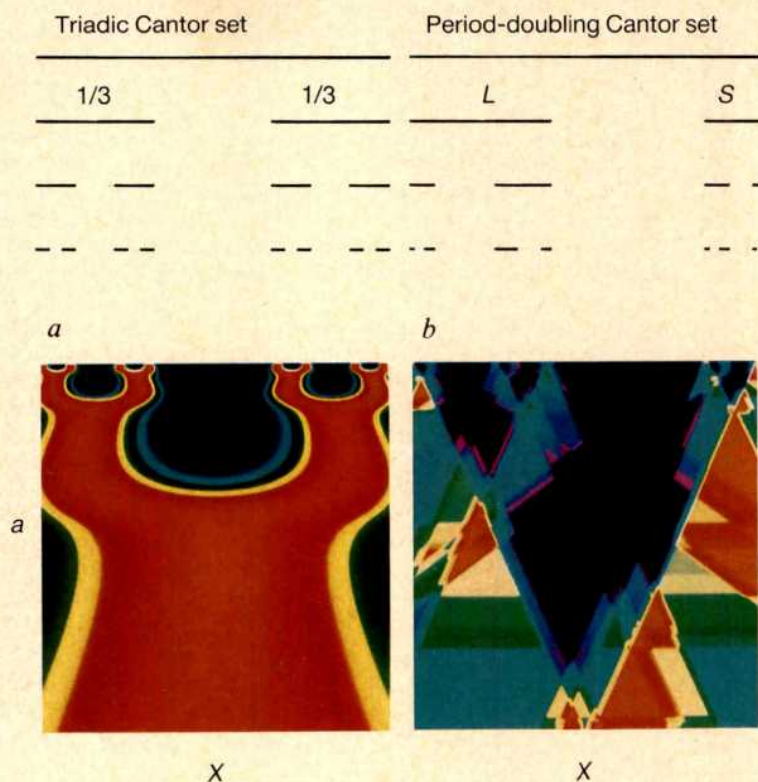


FIG. 1 Wavelet analysis of Cantor sets<sup>9,15</sup>. Coordinates correspond to space ( $x$ ) and scale ( $a$ ); both are linear (small scales at the top). The intensity of the wavelet transform is colour-coded according to the natural light spectrum from black (low intensity) ( $T_g \leq 0$ ) to red ( $\max(T_g) > 0$ ) on each line  $a = \text{constant}$ . *a*, Uniform triadic Cantor set analysed using a 'Mexican hat' wavelet:  $g(x) = (1 - x^2) e^{-x^2/2}$ ;  $x \in [0, 1]$ ,  $a \in [2^{-9}, 2^0]$ . *b*, Period-doubling Cantor set analysed using 'French hat' wavelets:  $g(x) = 1(|x| < 1)$ ,  $-1/2(1 \leq |x| \leq 3)$ ,  $0(|x| > 3)$ ;  $x \in [-0.40115, \dots, 1]$ ,  $a \in [2^{-10}, 2^{-1}]$ . Above each figure we illustrate the process of subdivision leading to each Cantor set.

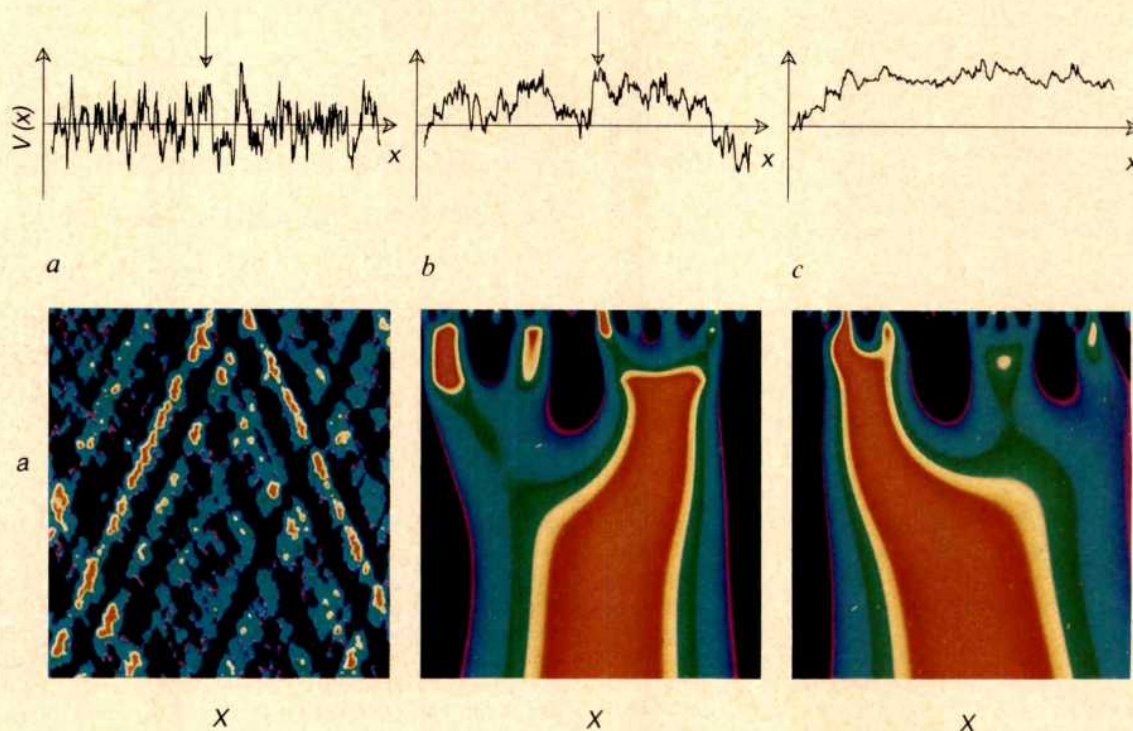


FIG. 2 Wavelet analysis of fully developed turbulence from wind-tunnel data. Coordinates and intensities are as in Fig. 1. The top graphs show the signals being analysed. Panel *a* is a 852-m-long sample, showing the large scales (from  $28l_0$  to  $l_0/10$ ) analysed using a 'French top hat' wavelet. In *b* the

'Mexican hat' wavelet was used for magnification  $\times 20$  of the central position indicated by the arrow in the top graph of *a*. Panel *c* bears an analogous relation to *b*, again using the 'Mexican hat' wavelet. The successive forkings reveal the fractal nature of the Richardson cascade.

fraction  $\beta$  of the eddies of smaller scales. More recently, a multifractal model of the fine-scale intermittency has been introduced by Parisi and Frisch<sup>5</sup>; this accounts for the more complex cascading process suggested by the experimental data on inertial-range structure functions obtained by Anselmet *et al.*<sup>6</sup>. These statistical models are insufficient, however, because they cannot give any topological information about the intermittent structures in real space. Here we use the wavelet transform<sup>7</sup> to analyse the velocity field of wind-tunnel turbulence at very high Reynolds numbers<sup>8</sup>. This 'space-scale' analysis is shown to provide the first visual evidence of the celebrated Richardson cascade<sup>1</sup>, and reveals in particular its fractal character<sup>3</sup>. The results also indicate that the energy-cascading process has remarkable similarities with the deterministic construction rules of non-homogeneous Cantor sets<sup>9,10</sup>.

Wavelet analysis<sup>7</sup> is a mathematical technique introduced recently for analysing seismic data and acoustic signals<sup>11,12</sup>. It provides a two-dimensional unfolding of one-dimensional signals, resolving both the position and the scale as independent variables. The method comprises an expansion of an arbitrary real-valued function  $s(x)$  over wavelets that are constructed from a single function  $g$  by means of dilatations and translations<sup>7,13</sup>. The wavelet transform of  $s(x)$  with respect to the wavelet  $g$  is defined as:

$$T_g(a, x) = w(a) \int g((x-y)/a) s(y) dy \quad a > 0, x \in \mathbb{R}$$

where the weight function  $w(a)$  is introduced for visual enhancement.

The wavelet  $g$  is a regular function, localized around  $x=0$ . For a large class of functions  $s(x)$ , the wavelet transform may be inverted, provided that  $g$  is admissible ( $\int g(y) dy = 0$ ) and satisfies others conditions<sup>7,14</sup>. The wavelet transformation can be regarded as a mathematical microscope<sup>9</sup>, for which position and magnification correspond to  $x$  and  $a^{-1}$ , respectively, and the performance of the optics is determined by the choice of the analysing wavelet  $g$ . Wavelet analysis has become a powerful tool for locating singularities<sup>9,10</sup>: a singularity of  $s(x)$  at  $x_0$  produces a cone-like structure in  $T_g$ , pointing towards the point ( $a=0, x=x_0$ ). The nature of the singularities may be inferred from the power-law behaviour of  $T_g(a, x=x_0)$  on increasing the magnification  $a^{-1}$ .

As emphasized by Arnéodo *et al.*<sup>9</sup>, the wavelet transform assists visualization of self-similar properties of fractal objects. In particular, it illustrates the complexity of the fractal under consideration, revealing the hierarchy that governs the relative positioning of the singularities. In Fig. 1a, the successive pitchfork branchings observed in  $T_g(a, x)$  as a result of increasing the magnification  $a^{-1}$  illustrate how the uniform (single-scale) triadic Cantor set is constructed<sup>9,10</sup>. The unit interval  $[0, 1]$  is initially divided into three sub-intervals, each of length  $l=1/3$ . The middle interval is removed and the same process is repeated on each of the two remaining sub-intervals, and so on *ad infinitum*. In Fig. 1b, the same analysis is performed for the multifractal (two-scale) Cantor set that arises at the onset of chaos through the period-doubling cascade scenario<sup>9,15</sup>. The pitchfork branchings observed on increasing the magnification  $a^{-1}$  are no longer symmetric, and occur not in pairs but successively, as the signature of two unequal scaling factors, one large and one small ( $L(\alpha_{PD}) \approx 1/2.5029 \dots$ ) and  $S(\alpha_{PD})$  respectively). Moreover, the internal self-similarity of  $T_g$  (which is central to the renormalization-group study of the period-doubling transition to chaos) illustrates the basic construction rule of the period-doubling Cantor set: from one stage to the next, the relative positions of the large and small sub-intervals is unchanged when dividing a small interval, but is exchanged when dividing a large interval.

The fractal structures proposed in fully developed turbulence are real-space structures, in contrast to fractal attractors, which reside in phase space. Thus the wavelet transform can be applied

directly to turbulent velocity fields obtained from experiment or from numerical simulations<sup>16</sup>. Here we report the first such analysis performed on the velocity data from high-Reynolds-number, three-dimensional turbulence. The data were obtained in the large wind tunnel S1 of ONERA in Modane, France. A hot wire probe was located on the axis of the return section which was 24 m in diameter and 150 m in length. The turbulence level, expressed by the ratio of the r.m.s. value of the longitudinal velocity fluctuations to the mean velocity, was less than 7%, which implies that time variations are equivalent to spatial variations (the Taylor hypothesis). The scale ratio of the velocity signal shown in Fig. 2 is about  $4 \times 10^4$ , giving 15 m for the integral scale  $l_0$  and 0.35 mm for the dissipation scale  $l_d$ . The Reynolds number based on the Taylor microscale is  $R_\lambda = 2,720$ . This turbulence has an inertial range of at least two decades<sup>8,17</sup>.

A signal of total length 852 m was analysed hierarchically. Figure 2a corresponds to the total span and to a range of scales (that is, of parameter  $a$ ) from  $28l_0$  to  $l_0/10$ . Figure 2b shows the region around the arrow in Fig. 2a, with position and scale variables magnified by a factor of 20, and Fig. 2c is related to Fig. 2b in the same way. A 'French top hat' piecewise constant analysing wavelet was used in the case of Fig. 2a, emphasizing the cone-like structures. A 'Mexican hat' wavelet was used in Figs 2b and c, because it provides a (marginally) better visualization of the Richardson cascade<sup>1</sup>. Only positive values of the wavelet transform are shown. In the figures the weight function  $w(a)$  was chosen such that the maximum of  $T_g(a, x)$  on each  $a = \text{const.}$  line is independent of  $a$ .

Large-scale eddies are revealed in Fig. 2a by the superimposed cone-like patterns of different intensities, indicating structures of scale  $a \sim l_0$ . These large-scale structures seem randomly distributed in space and are fairly space-filling. Very different small-scale patterns are displayed in Figs 2b and c. These are created by a process in which typically an eddy divides asymmetrically (in intensity) into two smaller eddies, and this subdivision is repeated about eight to ten times before eddies reach a scale at which they are readily dissipated. These successive forkings produce a fractal structure, in which small-scale activity becomes increasingly sparse. The ratio between the scales of successive generations can take different values, which is another indication that a multifractal description is appropriate.

In summary, we have used wavelet analysis, which is already known to be a powerful tool for analysing multifractal attractors<sup>9,15</sup>, to explore the spatial structure of fully developed turbulence. Our emphasis has been to identify new qualitative features. Similar techniques can be used to reveal fractal clustering of galaxies on very large scales (A. Bijaoui & E. Slezak, unpublished work). □

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# The 1988 US drought linked to anomalous sea surface temperature

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THE 1988 drought in the United States has been widely reported, not least as an indicator of the reality of the 'greenhouse effect'—see for example ref. 1. On the other hand, drought is a naturally occurring phenomenon. Here we have studied 30-day forecasts of the atmospheric flow over the United States using a complex numerical weather-prediction model initialized with data from May 1987 and May 1988. In addition, an experiment with 1988 initial conditions and 1987 sea surface temperatures was made. The results indicate that much of the difference between the 1987 and 1988 forecasts was associated with interannual variability in sea surface temperature. These results provide complementary evidence to that in ref. 2, which suggested that the US drought was linked to anomalous oceanic conditions in the tropical Pacific.

The El Niño event in the tropical Pacific ocean is now recognized as part of the natural variability of the coupled ocean-atmosphere system<sup>3</sup>. At its height, the El Niño event is associated with an anomalous warming of the surface waters of the east and central tropical Pacific. There have been many studies suggesting that in the northern winter, there is a link between this warming and unusual weather conditions in the extratropics<sup>4</sup>. During 1987 there was a moderate El Niño event. In 1988, however, conditions in the tropical east Pacific began to swing towards the opposite, 'anti El Niño' or 'La Niña', phase of the event where sea surface temperatures (SSTs) became anomalously negative. Little is known about the possible impact of La Niña in the extratropics.

The difference between SSTs averaged for the 15 days preceding 24 May 1987 and 22 May 1988 is shown in Fig. 1a. The relative warmth of the equatorial east Pacific in 1987 relative to 1988 (over 3 K), is clearly shown. To the north and south of this equatorial band, SST differences were reversed. It should be pointed out, however, that there were significant SST differences in other parts of the globe. As shown, differences of 3 K were also attained in the subtropical north Pacific, and differ-

ences of 2 K were observed in the Atlantic and Indian oceans. Whether these are causally linked to the El Niño cycle is unknown at present.

Figure 1b shows the observed time-average difference in the height of the atmospheric 300-mbar pressure surface between 25 May–23 June 1987 and 23 May–21 June 1988. Over much of North America the pressure surface was lower in 1987 than 1988. This is consistent with the fact that, during the 1988 drought, the jet stream and associated rain-producing weather systems were displaced northward of their usual positions by anomalous high pressure. Relative to 1988, the height of the 300-mbar surface was also depressed over a region extending from Alaska down to the subtropical north-east Pacific. Note also that there is a rather strong negative depression over the north-west Atlantic.

Based on observations of the anomalous height fields for 1988, and using theoretical concepts of Rossby-wave radiation from a tropical heat source, and a relatively simple numerical model, Trenberth *et al.*<sup>2</sup> suggested that the 1988 US drought and anomalous tropical Pacific SSTs were causally related, and inferred that the 1988 US drought was part of normal climatic variability.

The European Centre for Medium Range Weather Forecasts (ECMWF) has developed a complex and comprehensive global numerical weather-prediction model<sup>5</sup> which is integrated daily to give weather guidance up to ten days ahead. On an experimental basis over the past four years, the model has also been integrated to 30 days, twice a month, from initial data separated by 24 h. In general, the day-to-day variations of weather are not predictable beyond about two weeks into the future. However, from time to time, the 30-day extended range forecasts do show impressive skill (that is, the forecast and observations correlate well).

Figure 2 shows some 30-day mean difference fields from extended-range forecasts initialized on 24 May 1987 and 22 May 1988. Comparing the forecast 300-mbar height field difference in Fig. 2a with the observed height field difference (Fig. 1b), it can be seen that the difference in model simulations for the two years is quite skilful. In particular, the three depressions of the actual 300-mbar height surface described above, over the Atlantic, North America and the North-east Pacific, all have counterparts in the forecast field. Although the two fields clearly do not correlate perfectly, the verisimilitude of this extended-range forecast difference field is in general above average. Indeed the difference field for two 30-day forecasts, each initialized just

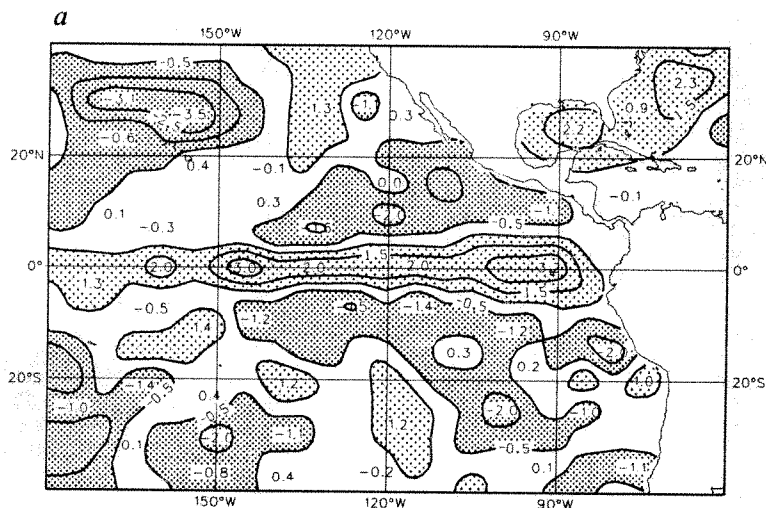
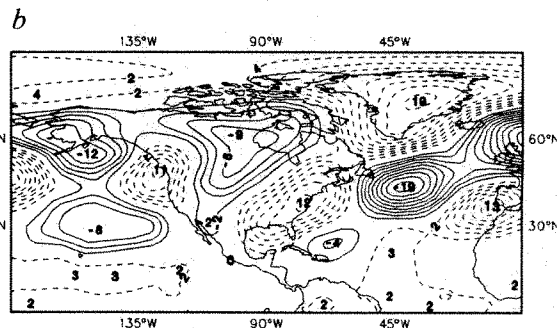


FIG. 1 a, Difference in sea surface temperatures between data averaged for 15 days preceding 24 May 1987, and for 15 days preceding 22 May 1988. Contour interval of 1 K. Areas above 0.5 K shown with coarse stipple; areas below -0.5 K shown with fine stipple. The two SST fields whose difference is shown here were used as oceanic boundary conditions for the model



integrations discussed in the text. Data from operational US National Meteorological Center analyses. b, Time-average difference in the height of the 300-mbar pressure surface between the 30 days following 24 May 1987 and the 30 days following 22 May 1988. Contour interval 20 cm. Positive values are shown with dashed contours.



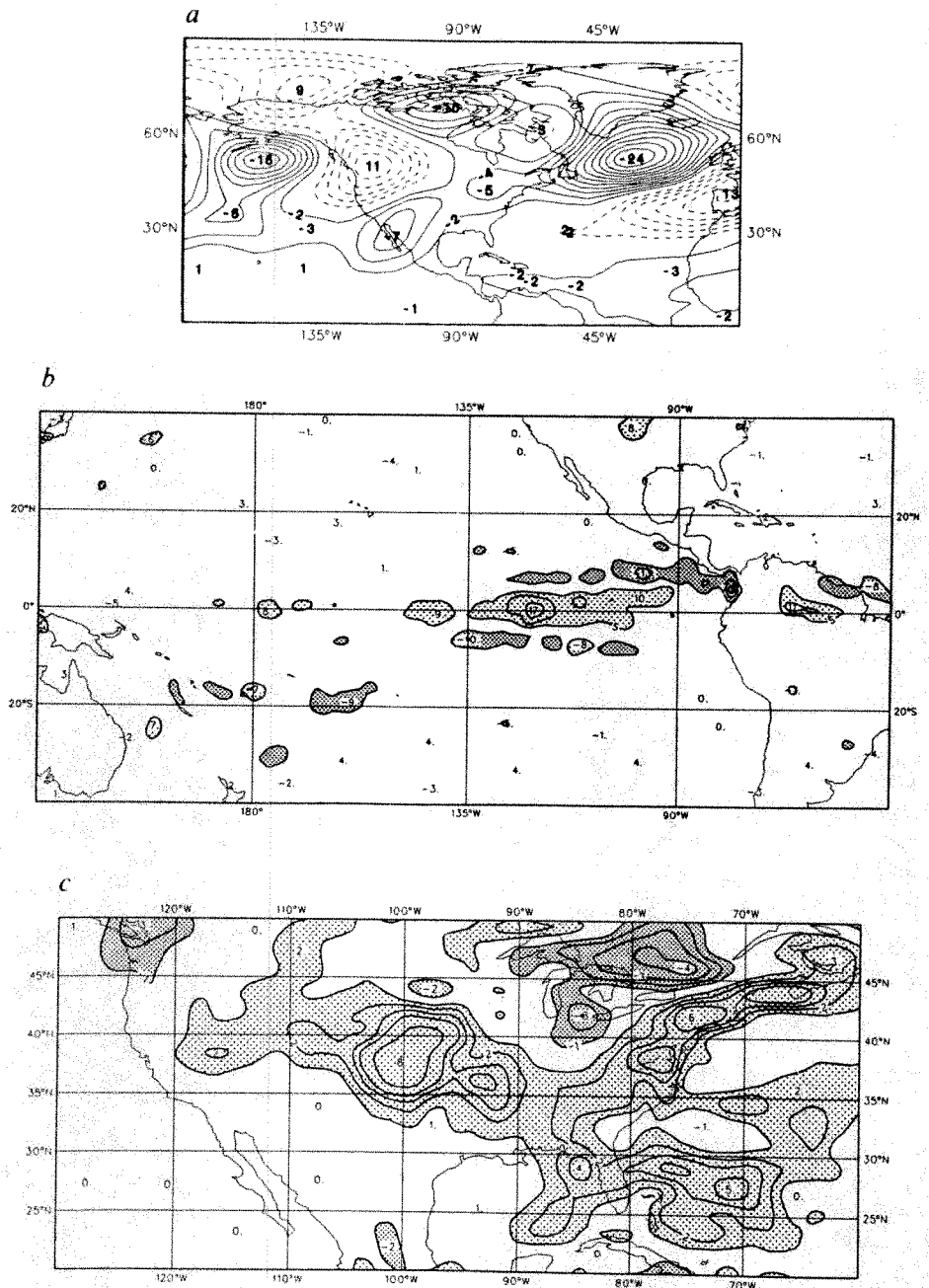


FIG. 2 Thirty-day average difference fields between two forecast integrations of the (T63) ECMWF numerical weather prediction model, the first initialized from data for 24 May 1987, the second from 22 May 1988. *a*, The height of the 300-mbar pressure surface, contours as Fig. 1*b*. *b*, Rainfall over the east tropical Pacific with contour interval of 5 mm per day (0 contour suppressed), coarse stippling above 5 mm per day, fine stippling below -5 mm per day. *c*, Rainfall over North America with contours of  $\pm 6$ ,  $\pm 4$ ,  $\pm 3$ ,  $\pm 2$ ,  $\pm 1$  mm per day; coarse stippling above 1 mm per day, fine stippling below -1 mm per day.

one day earlier than those shown here, was very much poorer. The sensitivity of extended-range forecasts to initial conditions can be expected on fundamental theoretical grounds<sup>6</sup>.

Figure 2*b* shows 30-day mean forecast difference rainfall fields over the tropical Pacific. Over the equatorial east Pacific there is a clear increase in rain in 1987 compared with 1988. To the immediate north and south, there is a relative decrease in 1987. This field is clearly correlated with the SST difference field shown in Fig. 1*a*, and agrees with satellite remote-sensing proxy observations of rainfall<sup>7</sup>.

Figure 2*c* shows 30-day mean forecast difference rainfall fields over North America. Over much of the eastern and central United States, the differences are generally positive and substantial, indicating in some areas a forecast of rainfall for 1988 less than 20% of that for 1987. Of course, these rainfall difference fields are not accurate in detail, but certainly capture much of the essence of the 1988 drought<sup>7</sup>.

The question arises as to whether the difference fields shown in Fig. 2 reflect primarily the fact that the initial conditions between the two integrations were different, or whether the

'boundary conditions' also played an important role. In numerical weather-prediction models (as opposed to many climate models), SSTs are specified and held fixed during an integration, and are therefore part of the boundary conditions for such models. For the ECMWF forecasts, SSTs are obtained as an average of observations for 15 days preceding the initial date. Figure 1*a* shows the difference field of SSTs used in the two forecasts.

To examine the importance of SST differences in accounting for the difference fields in Fig. 2, we have run a third 30-day integration using identical conditions to the 1988 forecast, but with SSTs from the 1987 integration. The difference in 30-day mean 300-mbar height and rainfall between this experiment and the 1988 forecast integration is shown in Fig. 3.

In testing the potential impact of SST anomalies on the atmosphere, it is common to run a single climate timescale integration of several years, or a large number of shorter timescale integrations from a variety of initial conditions. In the latter case, the impact of SSTs will in general depend quite strongly on the particular initial conditions<sup>8,9</sup>, and it may be

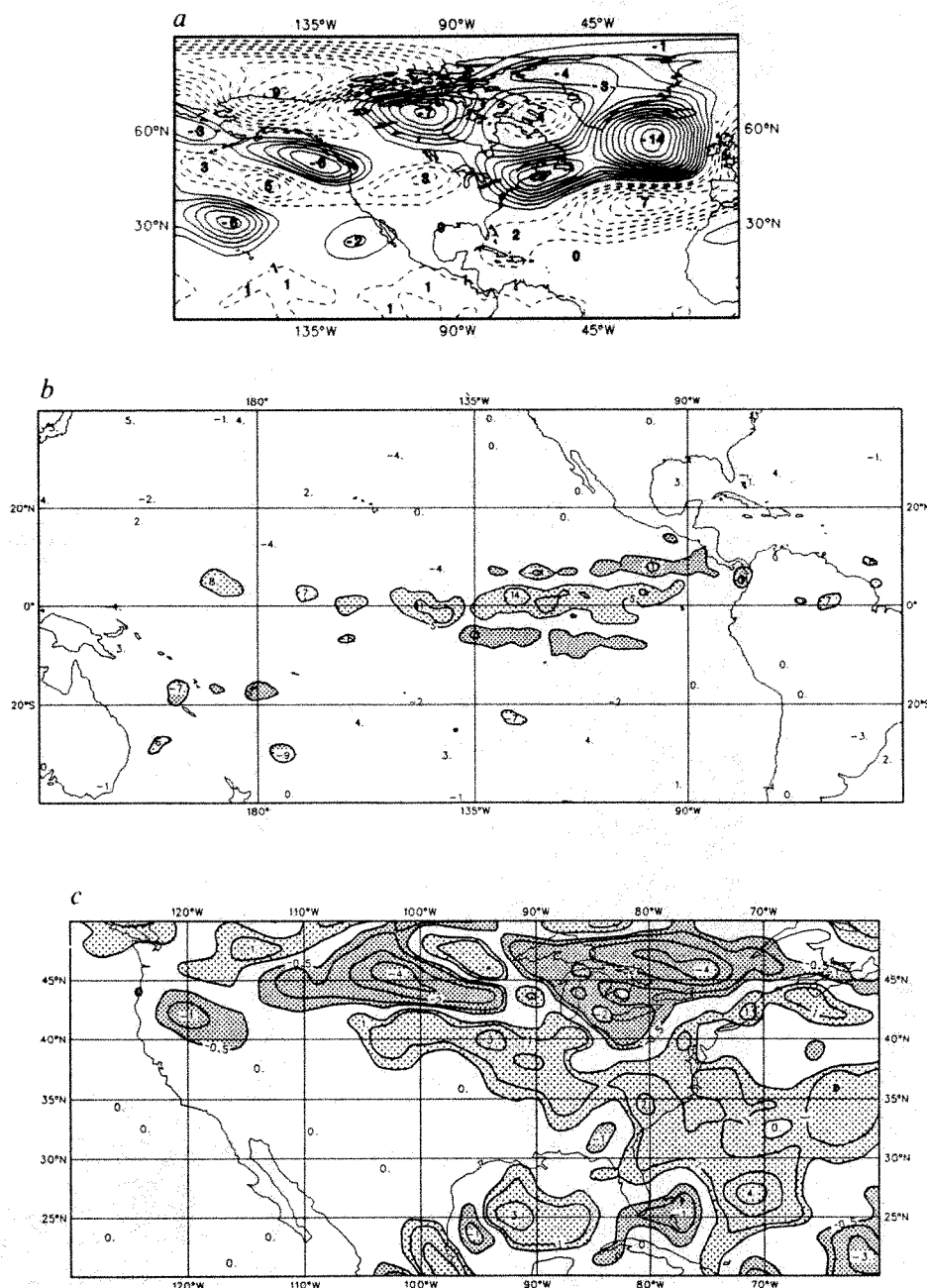


FIG. 3 Thirty-day average difference fields between two integrations of the (T63) ECMWF numerical weather prediction model; the first initialized from data for 22 May 1988 and sea surface temperatures from the 15 days preceding 24 May 1987, the second initialized from the same data but with sea surface temperatures averaged from the 15 days preceding 22 May 1988. *a*, The height of the 300-mbar pressure surface; contour interval 10 cm. *b*, Rainfall over the east tropical Pacific, contours as Fig. 2*b*. *c*, Rainfall over North America with contours of  $\pm 5$ ,  $\pm 3$ ,  $\pm 2$ ,  $\pm 1$ ,  $\pm 0.5$  mm per day; coarse stippling above 0.5 mm per day, fine stippling below  $-0.5$  mm per day.

that one would deduce that the response of the atmosphere to a particular SST anomaly was not strongly significant. If one has the option, however, of verifying the shorter-timescale integrations as forecasts, one finds that many, if not most, show little extended-range forecast skill, although a small (maybe very small) fraction show exceptional extended-range skill. The question then arises as to whether the response of the unskilful forecasts to the imposed SST anomalies should be given the same weight as the response to the skilful forecasts (as would conventionally be the case). We suggest that they should not. This is the reason why we choose to emphasize the response to our skilful forecast, rather than the unskilful forecast initialized one day earlier. (In fact one could broadly think of our results in terms of an ensemble mean of two forecast experiments, weighted *a posteriori* by the extended-range skill of the basic forecast.)

The 300-mbar height difference field (Fig. 3*a*) is clearly correlated with the forecast difference field shown in Fig. 2*a*, although the amplitude of the field is smaller (note the reduced contour interval in Fig. 3*a*). Note, in particular, the depression of the

height of the 300-mbar surface over the Atlantic and over North America. The depression over the North Pacific is certainly not as strong in Fig. 3*a* as in Fig. 2*a*, although the general pattern of wind anomalies that would result from these height field differences corresponds well.

Rainfall differences over the tropical Pacific are shown in Fig. 3*b*. Comparing with Fig. 2*b*, there can be no doubt that the simulated difference between 1987 and 1988 rainfall in that region is primarily the result of the different boundary conditions.

Rainfall differences over North America are shown in Fig. 3*c*. It can be seen that there is a general increase in rainfall with 1987 SSTs to the south of  $40^\circ$  N, and the pattern is well correlated with the forecast difference in Fig. 2*c*. Moreover, there is a decrease in rainfall over the Great Lakes area, consistent with the decrease there in Fig. 2*c*. Again, it should be emphasized that Fig. 3 should be compared with Fig. 2 rather than the observations; shortcomings in the veracity of the forecast fields are reflected in similar shortcomings for the experiment.

These results clearly indicate that the impact of the SSTs was

in the same sense as the difference between the two forecasts. The amplitudes in Fig. 3 are clearly smaller than in Fig. 2, because the impact of the SSTs is not felt to any significant extent in the first few days of the forecast.

Hence we conclude that the anomalous SSTs in 1987 and 1988 were indeed important in accounting for the reduction in rainfall over the United States in the late spring of 1988. We cannot, on the basis of these results, state that La Niña itself (rather than other regions of SST anomaly) was principally important. Further experiments should help to clarify this. However, on the basis of this study and the investigation of Trenberth *et al.*<sup>2</sup>, using very different techniques to those discussed here, tropical Pacific ocean temperatures related to the El Niño/La Niña event cycle appear to be important, and there now seems to be mounting evidence that the 'cause' of the 1988 US drought was primarily associated with phenomena that are part of the natural variability of the climate system. □

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## Organic geochemical evidence for global fires at the Cretaceous/Tertiary boundary

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MANY hypotheses have been advanced to explain the mass extinction at the Cretaceous/Tertiary (K/T) boundary<sup>1–3</sup>. Recently, Wolbach *et al.*<sup>4</sup> suggested that massive forest fires were triggered by the impact of a meteorite, and cite as evidence the presence of elemental carbon (mainly soot) from K/T boundaries<sup>5</sup>. Almost all of the airborne polycyclic aromatic hydrocarbons (PAHs) generated by pyrosynthesis are adsorbed, through hydrogen bonding, on the surface of soot, the particulate fraction from combustion<sup>6,7</sup>. Although soot itself is a polymer of polybenzenoid radicals, early termination of polymerization leads to enhanced PAH production. Pyrosynthesis of PAHs is thus favoured by a chemically reducing atmosphere. If there were wildfires, a group of high-molecular-weight parent PAHs characteristic of combustion, predominating over their alkyl homologues<sup>8,11</sup>, should be present in K/T boundary samples known to contain soot<sup>4,5</sup>. Here we compare K/T samples from New Zealand, Italy and Denmark to those from above and below the boundary, and find enhanced PAH contents and distribution profiles that reflect a pyrolytic origin. The data thus provide the first detailed organic-molecular evidence for the combustion source of organic carbon at the K/T sites.

The organic-rich clay layer (corresponding to S6 to S10 of Schmitz<sup>12</sup>) above the red layer was collected by B. Schmitz by digging into the Fish Clay outcrop at Stevns Klint, Denmark (200 m south of the Højstrup Church). Only the unweathered clay was gathered. Woodside Creek boundary clay (upper layer) and two samples, one well into the Tertiary and the other well into the Cretaceous, were obtained from R. R. Brooks (Table 1). The exact location, stratigraphy and other details of depositional environment are available<sup>13,14</sup>. The red boundary clay, and a sample from above and below the boundary, from Bottaccione Gorge, Gubbio, Italy, were collected by W. Cavazza.

Sample location and trace-element distribution are described in ref. 15. The samples were shipped by air in sealed plastic bags, some first wrapped in aluminium foil.

Chunk samples were quickly sonicated in dichloromethane to remove external contamination. Dry powdered samples were then extracted in dichloromethane and fractionated into aliphatic and aromatic hydrocarbons and other classes of compound according to the methods of Venkatesan *et al.*<sup>16</sup>, which involve thin-layer and column chromatography. The aromatic hydrocarbon fraction (containing PAHs) was analysed by gas chromatography and quantified by gas chromatography/mass spectrometry (GC/MS), based on the response factor of an internal standard (dodecylbenzene) and an external standard mixture containing PAH compounds listed under Fig. 1.

Pertinent geochemical data are presented in Table 1. The iridium enrichment at the boundary clays is generally concomitant with the enhanced levels of organic carbon and total PAH content. This is also consistent with a recent observation<sup>17</sup> that the bulk of the iridium is probably organically complexed.

The distribution of PAHs in K/T extracts is illustrated in Fig. 1. The Stevns Klint and Gubbio samples are enriched in high-molecular-weight PAHs relative to the Woodside Creek sample. Phenanthrene is the most dominant and 2-methylfluorene the second most dominant PAH in the Woodside Creek sample. In the Stevns Klint sample, coronene is the most abundant PAH, followed by phenanthrene. Coronene is also the most dominant PAH in the Gubbio sample, followed by benzo(*b+k*)fluoranthene and chrysene/triphenylene. Pyrene and fluoranthene are significant constituents in the Stevns Klint and Gubbio samples. The dominant analogues in both of these samples are the peri-condensed aromatic series, such as pyrene, benzo-pyrene, benzo(*g,h,i*)perylene and coronene. These peri-condensed compounds are formed by the pyrolysis of organic matter<sup>18</sup> such as gasoline<sup>19</sup> and wood<sup>20</sup>, and are relatively uncommon in petroleum<sup>6</sup> but have been detected in significant amounts in hydrothermal mounds<sup>21</sup>. Because peri-condensed structures are more reactive than their angularly-condensed analogues<sup>18</sup>, the presence of the former in significant amounts in these samples implies that rapid thermal quenching by adsorption of these PAHs in soot contributed to their stability. Also, the abundance of these pyrolytic PAHs should have been high compared with that seen today, the latter representing the residual combustion PAHs after degradation<sup>19</sup>.

The absence of peri-condensed PAHs in the Woodside Creek K/T sample could be attributed to one of the following. (1) They were not formed because there were no wildfires. (2) The continent adjacent to this site experienced elevated temperatures for longer intervals relative to the other two sites, with

TABLE 1 Pertinent geochemical data for K/T site samples

Sample	Total % organic carbon	Ir (p.p.b.)†	Total PAH* (p.p.b.)
Woodside Creek, New Zealand			
K/T	0.72	26‡	33
K/T + 250 cm	0.02	NA	5
K/T – 275 cm	0.01	NA	3
Stevns Klint, Denmark			
K/T	1.40	50	30
Gubbio, Italy			
K/T	0.10	5§	75
K/T + 5–20 cm	NA	NA	<1
K/T – 3–15 cm	NA	NA	<1

NA, Not available.

\* Total PAH summed from naphthalene to 1, 2, 4, 5-dibenzopyrene listed under Fig. 1.

† Parts per 10<sup>9</sup>.

‡ Personal communication from R. R. Brooks.

§ Data from ref. 15.



consequent elimination of the less stable, intermediate<sup>18</sup>, peri-condensed PAHs. No literature data is available at present on the stability of peri-condensed PAHs and their burial history. The presence of five-membered alicyclic rings such as 2-methylfluorene, 2,3-benzofluorene and fluoranthene also attest to the pyrolytic origin of PAHs in all three of the sites. These PAHs are commonly present in all pyrolysates from organic matter and, once formed, are not easily converted to peri-condensed aromatics, because they inhibit subsequent enlargement of the aromatic nuclei<sup>18,22</sup>. Thus the chemical composition of the PAH fraction suggests a pyrolytic source. The Woodside Creek K/T sample, therefore, reflects input of PAHs from combustive sources, possibly from an adjacent land mass that experienced extended periods of high temperatures caused by forest fires, resulting in the destruction of less stable, linear PAHs<sup>18</sup>. (3) The Woodside Creek site shows considerable lateral variations in boundary-clay thickness and Ir abundances, which are apparently also reflected in the PAH profile. This may explain the relatively high level (1.4 parts per 10<sup>9</sup>) of coronene reported earlier from a Woodside Creek K/T layer<sup>23</sup>.

The dominance of parent PAHs is characteristic of combustion products, whereas alkylated PAHs predominate in bitumen and petroleum<sup>8-11,22</sup>. The predominance of parent PAHs in the boundary samples (Fig. 2) indicates that they have been subjected to far higher temperatures than those involved in petroleum formation. On the other hand, the two samples above and below Woodside Creek K/T boundary are enriched in alkyl

homologues and exhibit patterns characteristic of petroleum<sup>8-11,22</sup>. However, the effects of biodegradation and water washing<sup>24</sup> and weathering<sup>25</sup> necessitate caution in interpreting the PAH data. Data from other lipid components indicate that both the Stevns Klint and Woodside Creek samples are biodegraded, although not to the extent of total removal of *n*-alkanes (J. D. *et al.*, preprint). Weathering could cause the removal of alkylated PAHs<sup>25</sup>, resulting in a pyrolytic profile. However, comparison of the unresolved complex mixture of aliphatic-fraction and normal-alkane profiles from Woodside Creek indicates that the three samples (K/T, +250 cm, -275 cm) apparently were subjected to similar extents of weathering (J. D. *et al.*, preprint; M. I. V., unpublished data). Yet samples above and below the boundary exhibit PAH alkyl homology significantly different from that at the boundary, suggesting that the boundary experienced an input of combustion PAHs significantly above the background level. It is also known that parent PAHs are more susceptible to biodegradation than are the alkyl PAHs and that the rate of biodegradation decreases with increasing degree of alkylation under oxic conditions<sup>24</sup>. Assuming that some biodegradation has occurred, the elevated abundance of parent PAHs relative to alkyl PAHs at the boundary provides convincing evidence for an input of combustion material to the boundary layer.

Alkyl homology at the boundary is similar to that in the profiles reported from soot<sup>11</sup> and recent sediments receiving airborne combustion inputs<sup>8-11</sup> (Figs 2 and 3). The alkylation

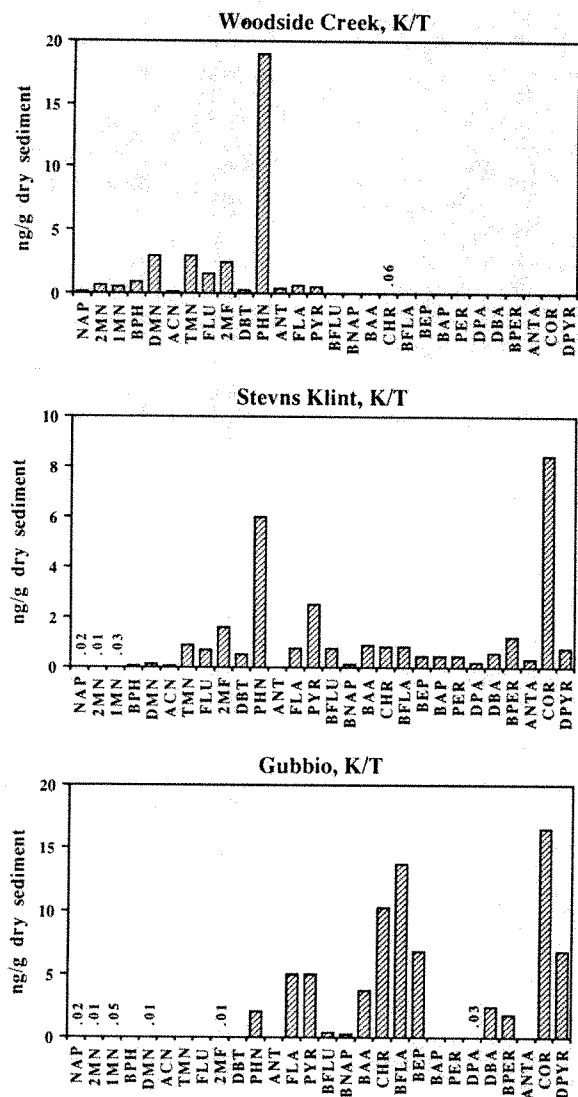


FIG. 1 PAH distribution at the K/T boundaries. The following compounds in the external standard mixture were quantified: naphthalene (NAP); 2-methylnaphthalene (2MN); 1-methylnaphthalene (1MN); biphenyl (BPH); 2,6-dimethylnaphthalene (DMN); acenaphthene (ACN); 2,3,5-trimethylnaphthalene (TMN); fluorene (FLU); 2-methylfluorene (2MF); dibenzothiophene (DBT); phenanthrene (PHN); anthracene (ANT); fluoranthene (FLA); pyrene (PYR); 2,3-benzofluorene (BFLU); 1,1'-binaphthalene (BNAP); benzo(a)anthracene (BAA); chrysene/triphenylene (CHR); benzo(b+k)fluoranthene (BFLA); benzo(e)pyrene (BEP); benzo(a)pyrene (BAP); perylene (PER); 9,10-diphenylanthracene (DPA); 1,2,5,6-dibenzanthracene (DBA); benzo(g,h,i)perylene (BPER); anthanthrene (ANTA); coronene (COR); 1,2,4,5-dibenzopyrene (DPYR). Numbers printed above some compounds refer to concentrations too low to be plotted. Quantification was carried out with a Finnigan Model 4000 quadrupole mass spectrometer interfaced directly with a Finnigan Model 9610 gas chromatograph. A fused-silica DB5 (J&W) column of 30 m  $\times$  0.25 mm was programmed from 35  $^{\circ}$ C to 290  $^{\circ}$ C at 4  $^{\circ}$ C min<sup>-1</sup> and then held at constant temperature for  $\sim$ 2 hours. Helium was used as a carrier gas; electron energy was 70 eV; source temperature was 240  $^{\circ}$ C; scan speed 2 s per scan from 50 to 550 AMU. Analyses were performed in the full scan mode and the extracted-ion-current profiles were used in the identification and quantification. The PAH fraction was also screened for substitution by alkyl homologues up to C<sub>4</sub> to C<sub>5</sub> (from NAP to PER), in addition to those listed above.

patterns of K/T samples from Woodside Creek and Gubbio are characteristic of combustion products of wood or kerosene, whereas the Stevns Klint profile is more akin to that from combustion of coal<sup>22</sup>. These patterns can originate from combustion at temperatures of 400–800 °C (as occur during forest fires), which could allow some alkylated PAHs to survive while thermally cracking extended chains into radicals, which recombine to form larger aromatic systems by pyrosynthesis<sup>8</sup>.

Based on the detection of retene (1-methyl-7-isopropyl-

phenanthrene) in Woodside Creek<sup>5</sup> and in the DSDP site 605 (ref. 26), biomass is considered a strong candidate as the fuel for such a fire<sup>5</sup>. We detected retene and simonellite at  $<1$  part per  $10^9$  in the K/T samples, indicating that although biomass may have been a fuel source, the retene concentration is too low to confirm this. Alkylated phenanthrenes have indeed been detected in volcanic ash from Mt St Helens as a result of pyrolysis of plant material<sup>27</sup>, and in emissions from wood combustion<sup>20</sup>. The yield of retene apparently depends on oxygen availability

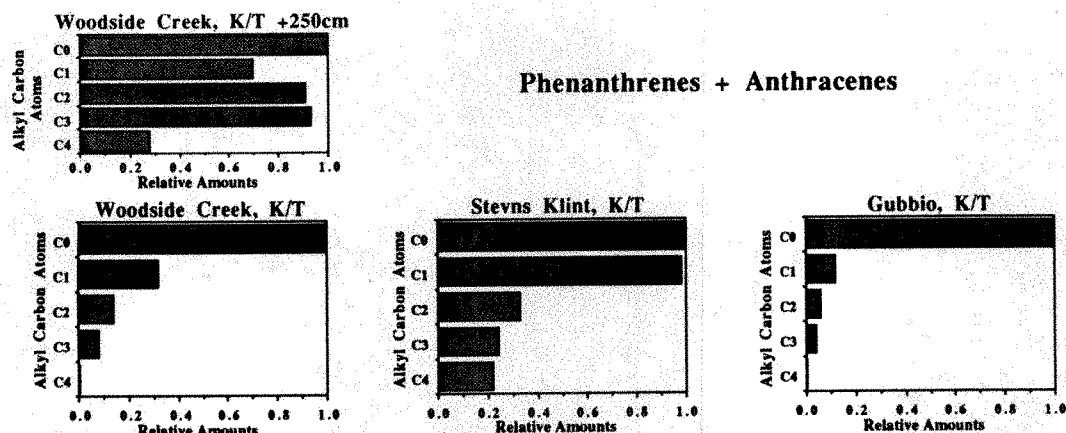


FIG. 2 Alkyl homologue distributions of selected PAHs. The most abundant homologue in each sample is set to 1.0.

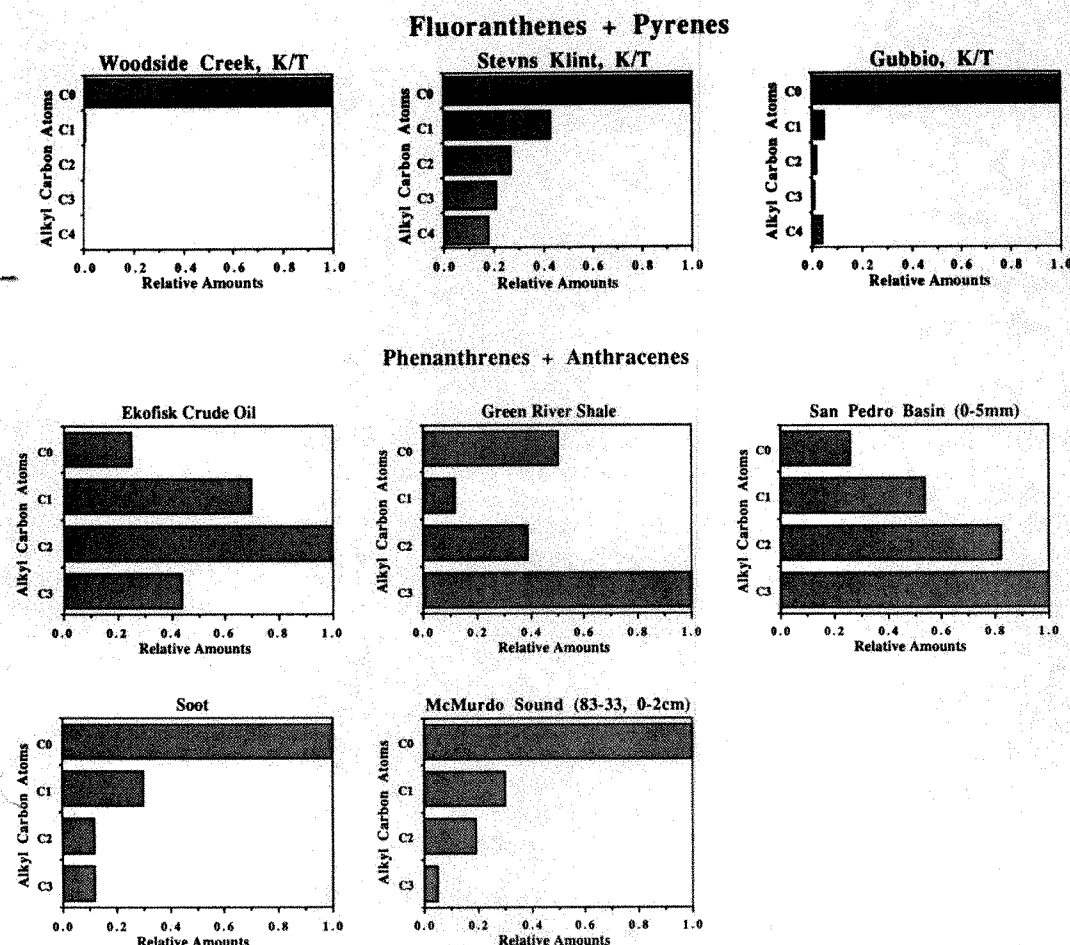


FIG. 3 Alkyl homologue distributions of phenanthrenes + anthracenes plotted from selected literature data. Note the enrichment of alkylated PAHs in oil<sup>24</sup>, shale and recent, petroleum-contaminated sediment from San Pedro Basin, southern California Bight<sup>10</sup>. Recent sediment from McMurdo Sound<sup>28</sup>, Antarctica, illustrates the combustion PAH profile, similar to that found in soot. Airborne combustion characteristics of PAHs in the Antarctic sediments are described in ref. 29. Soot data are plotted from ref. 11. Note the similarity of K/T boundary samples to McMurdo Sound PAH profile, and those of the Woodside Creek K/T +250 and -275-cm to the San Pedro Basin PAH profile.

and is inversely proportional to temperature<sup>20</sup>. The relatively low ratio of retene to other PAHs (such as phenanthrene) in the K/T boundary clays is consistent with moderate- to high-temperature (400–800 °C) pyrosynthesis<sup>6</sup>. However, the low amounts of retene could also reflect a contribution from diagenesis of dehydroabietic acid<sup>28</sup>, which would bring into question a thermal origin of retene from biomass fires.

In conclusion, our data suggest that the PAH distributions at the K/T boundary from Woodside Creek, Stevens Klint and Gubbio are characteristic of a combustion origin, which is consistent with the earlier suggestions of massive global fires<sup>4,5</sup>.

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## Graphitized diamonds from a peridotite massif in Morocco and implications for anomalous diamond occurrences

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**DIAMONDS** are found in several areas of the world where their currently accepted source, kimberlite/lamproite volcanism, has not been found. Garnet pyroxenite layers in the Beni Bousera peridotite massif, northern Morocco, contain octahedral and other cubic forms of graphite, which we interpret here as pseudomorphs after diamond. This occurrence demonstrates that fragments of highly diamondiferous mantle are tectonically emplaced into the continental crust, providing an alternative source for some diamonds.

The Beni Bousera peridotite massif forms part of the Betico-Rifean Fold Belt. A continuous gravity high between Beni



FIG. 1 Photograph of upper half of a sharp-edged octahedron protruding from the garnet pyroxenite layer.

Bousera and the Ronda peridotite in south-west Spain<sup>1</sup> indicates that the Betics and Rif are part of the same tectonic system. Both the Beni Bousera and Ronda massifs were emplaced into the crust at high temperature during the Alpine orogeny<sup>2,3</sup>. In addition to peridotites, both massifs contain many mafic layers of varying composition. The predominant rock type within the Beni Bousera peridotite massif is a variably altered spinel lherzolite accompanied by rare harzburgites and dunite pods. Mafic layers form up to 10% of the outcrop<sup>4</sup>. Four garnet clinopyroxenite layers are known to contain graphite octahedra<sup>5</sup> (Fig. 1). The graphite pyroxenite layers comprise orange pyrope almandine garnet and omphacitic pyroxene porphyroclasts with minor amounts of plagioclase, spinel and sulphides. Graphite forms up to 15% by volume of the layers, and never cross-cuts the layer margins into the surrounding peridotites, which significantly contain no disseminated graphite. Graphite is also found in late-stage, low-temperature Cu–Ni mineralized veins which cross-cut the emplacement fabric of the massif. The graphite–garnet clinopyroxenites are not associated with this mineralization.

Graphite forms liberated by acid dissolution are multicrystalline assemblages of small graphite crystallites, here termed aggregates. They consist of (1) sharp-edged octahedra, with or without rounded fibrous graphite coats (the most abundant form); (2) rhombicuboctahedra<sup>6</sup> exhibiting well formed cube and octahedral faces; (3) contact twins or macles, with and without re-entrant angles, most of which are coated; (4) irregular to rounded masses whose surface morphology resembles framesite (phanerocrystalline carbonado<sup>7</sup>); and (5) octahedra that have been flattened and/or sheared along the {111} faces.

Graphite with no regular symmetry or form is widespread and probably represents highly deformed cubic forms. The regular crystalline forms of this graphite belong to the cubic system whereas natural graphite crystallizes in the hexagonal system. Graphite could be pseudomorphic after several cubic minerals. The most plausible geologically are diamond and spinel group minerals such as chromite and magnetite. Sub-millimetre anhedral spinel grains do occur in some mafic layers in Beni Bousera (less than 1% by volume). However, the relatively low Cr content (450–1,000 p.p.m.) of the bulk rock, and the low Cr and Fe<sup>3+</sup> content of the silicate minerals, are not consistent with the rock having once contained up to 15% of either chromite or magnetite. Additionally, petrographic studies show no evidence of either mineral as relicts within the graphite aggregates, and ashed graphites<sup>5</sup> confirm their absence.

All the regular crystalline forms of the graphite are displayed by natural diamond. Scanning electron microscopy (SEM) reveals that {111} faces of the graphite octahedral cores display pronounced steps which are comparable to the {111} growth



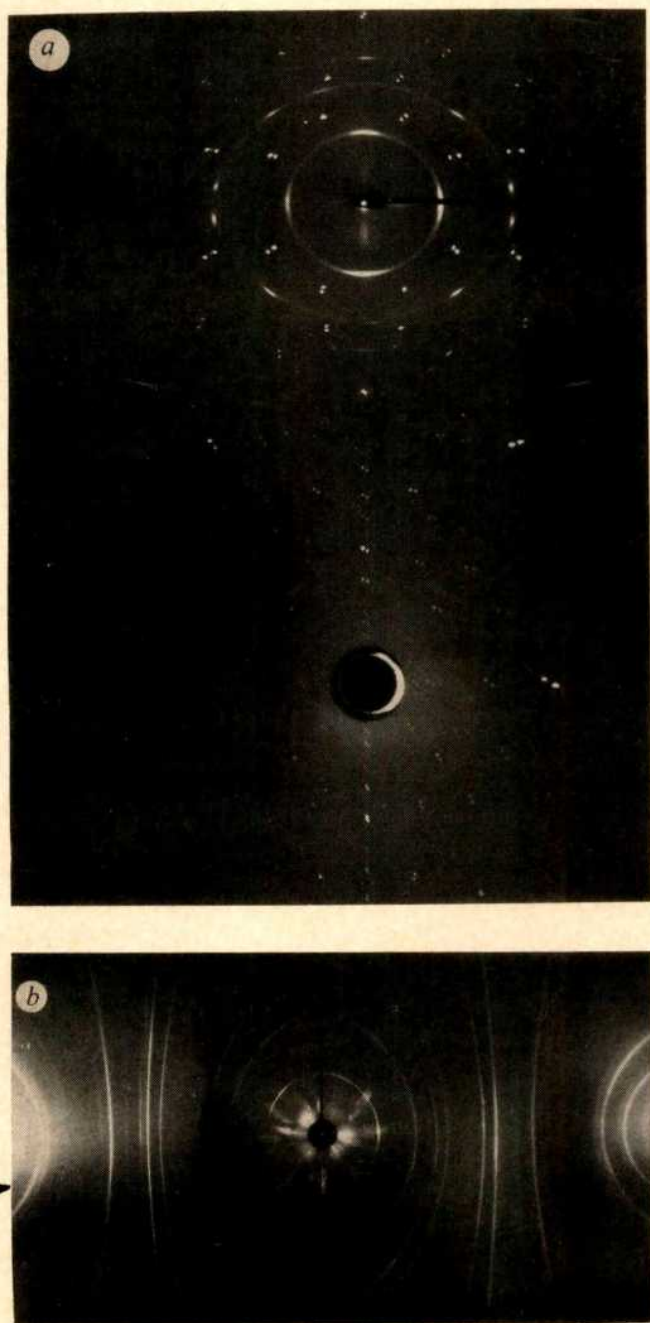


FIG. 2 *a*, Laue photograph of experimentally graphitized diamond, showing the strongly preferred orientation of graphite [0001] parallel to diamond {111} (courtesy of G. Cooper). Cu  $K\alpha$  radiation. [110] axis vertical; beam along [001]. *b*, Laue photograph of Beni Bousera graphite octahedron, showing preferred orientation of graphite [0001] parallel to {111} of the octahedron. Cu  $K\alpha$  radiation. The [110] axis is vertical; beam positioned to show the expected enhancement of graphite (0002) on the zero layer assuming it is parallel to {111}.

plates observed on natural diamond. Also evident on the {111} faces are small pits of trigonal symmetry, which have similar geometry and orientation to the etched trigons on the faces of diamond from volcanic pipes. In addition, SEM observations demonstrate that the basal {0001} planes of graphite crystals in the core are approximately parallel to the {111} faces of the octahedra. In contrast, graphite crystals in the fibrous coat are much more irregular in their orientation, with their basal planes at a high angle, often perpendicular, to the {111} surfaces of the core. X-ray diffraction<sup>8,9</sup> (G. Cooper, personal communica-



FIG. 3 Scanning electron photomicrograph of a clinopyroxene inclusion within a graphite octahedron, displaying apparent cubo-octahedral faceting. The {100} and {111} facets of the inclusion are parallel to the {100} and {111} faces of the octahedron.

tion) reveals that the graphite produced by experimental graphitization of natural diamond between 4.8 and 10 GPa pressure produces graphite crystallites whose {0001} planes lie parallel to the {111} faces of the original diamond octahedra (Fig. 2*a*). Single-crystal X-ray diffraction patterns of the octahedral graphite forms from Beni Bousera sometimes show a strong preferred orientation similar to that observed on experimentally graphitized diamond (Fig. 2*b*).

Graphite exhibiting cubic morphology has been found previously only in extraterrestrial samples, in the form of cliftonite<sup>12</sup>. In these cases the multicrystalline graphite aggregates take the form of cubes, cubo-octahedra and pseudo-hexagonal outlines. These aggregates do not display the preferred orientation noted above. The sixfold axis of the graphite lies parallel to the [001] and [113] axes of the cliftonite<sup>13,14</sup> and thus these examples are not considered to be pseudomorphs after diamond<sup>13,14</sup>. Therefore, graphite pseudomorphing a cubic mineral will not necessarily acquire the preferred orientation seen in the Beni Bousera graphite.

Clinopyroxene and garnet are both observed as inclusions within octahedra in thin section. Radiographs indicate that these inclusions in the graphite octahedra occasionally occupy a large proportion of the interior. Some inclusions possess well formed cubo-octahedral facets identical to many inclusions within natural diamond<sup>10,11</sup> (Fig. 3). The mechanism by which diamond imposes cubic symmetry on minerals of lower symmetry, such as monoclinic pyroxene, is not clearly understood. However, this phenomenon is powerful evidence for a diamond precursor for the Beni Bousera graphite aggregates.

The Beni Bousera octahedral graphite aggregates display a sharp physical boundary between cores of cubic symmetry with regular crystallites, and rounded fibrous coats. In general, there is a difference in style of deformation between core and coat. Core graphite exhibits relatively coarse sub-grains when cut parallel to a cube axis, whereas the fibrous-coat graphite possess kink lamellae. These differences are due to the marked mechanical anisotropy of graphite, deformation style depending on the orientation of graphite basal planes with respect to the direction of principal stress. Raman spectroscopy (J. D. Pasteris, unpublished data) indicates that both coat and core graphite are extremely well crystalline and hence of high-temperature origin. The graphite aggregates are thus similar to coated diamonds; the latter are characterized by well formed cores, often clear octahedra, surrounded by diamond displaying a fibrous, radial growth, giving rise to the generally rounded



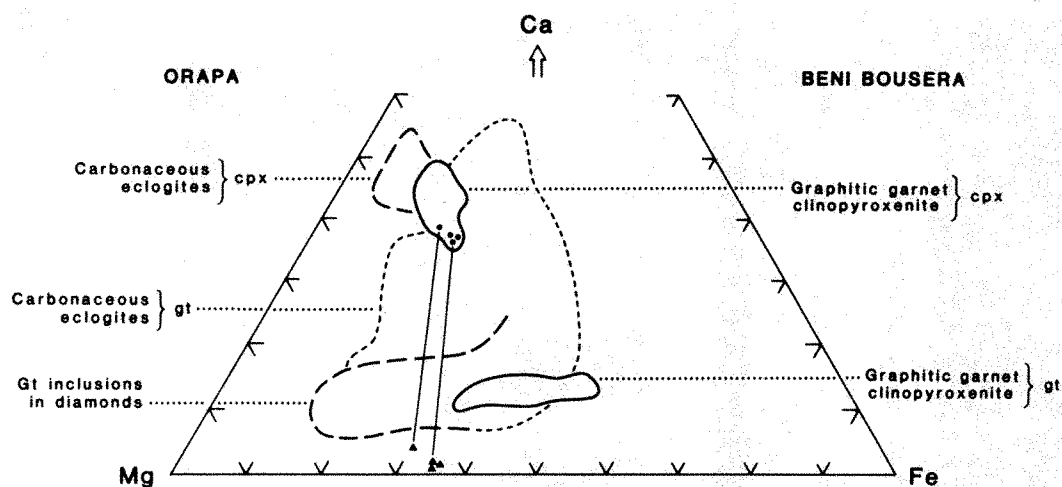


FIG. 4 Ternary Ca-Mg-Fe atomic-proportion plot of coexisting garnet-clinopyroxene-orthopyroxene in graphite garnet clinopyroxenites. The data are illustrated as fields, for clarity. Circles denote clinopyroxene, triangles denote orthopyroxene. Tie lines join coexisting phases of clinopyroxene and orthopyroxene. Fields for Orapa carbonaceous eclogites and inclusions in diamonds are shown<sup>20</sup>.

external morphology of thickly coated diamonds<sup>15,16</sup>. We conclude, therefore, that the graphite present in the garnet pyroxenites is the product of graphitization of diamonds, many of which may have been coated stones of some type.

The coats of coated diamonds are thought to be the result of rapid precipitation of diamond around a pre-existing nucleus associated with kimberlite genesis and eruption<sup>18</sup>. Our evidence may indicate that not all coated diamonds originate in this way.

The largest graphite aggregate consists of an octahedral core of edge length 12 mm surrounded by a coat 2–3 mm thick, such that the whole aggregate would be approximately equivalent to 10 carats if it were diamond. If all the graphite within the graphite-bearing garnet pyroxenite layers is assumed to be pseudomorphic after either macro- or micro-crystalline diamond, then the original diamond concentration of these layers would have been 15% by volume, that is, more than 10<sup>5</sup> times richer than the concentration found in high-grade kimberlites and lamproites but similar to concentrations in some small diamondiferous eclogite xenoliths<sup>18,19</sup>.

Major-element chemistry of graphitic and non-graphitic garnet clinopyroxenites is similar other than in respect of carbon contents. Compositions are also comparable to those of eclogite xenoliths found in kimberlite, being of picritic basalt composition (10.75–16.33% MgO) but having slightly lower total content of alkaline metals. The garnetiferous rocks from Beni Bousera, however, have undergone more extensive re-equilibration than have typical eclogite xenoliths, such that a significant proportion of the garnet has exsolved from clinopyroxene. Clinopyroxenes are aluminous sodic-augite to omphacite (up to 11.8 wt % Al<sub>2</sub>O<sub>3</sub>, 23 mol % jadeite) and fall within the field defined by carbonaceous eclogites<sup>20</sup> (Fig. 4). Garnets (Py<sub>57–34</sub>, Al<sub>48–29</sub>, Gr<sub>12–8</sub>) are within the large range measured from diamond-bearing eclogite xenoliths<sup>20</sup>.

Because of the essentially biminerallitic nature of the garnet pyroxenite layers, it is not possible to establish the pressures at which they last equilibrated. But microprobe studies using wavelength-dispersive methods demonstrate that despite substantial re-equilibration some garnets retain a slight excess of silica and small amounts of sodium (0.05 wt %). These compositions are comparable to those found in diamond-bearing eclogites and are a consequence of high-pressure pyroxene solid solution in garnet<sup>21</sup>, which becomes significant only at ~50 kbar pressure (depths of ~150 km), that is, within the stability field of diamond.

All of these lithologies contain evidence of lower-pressure re-equilibration. Garnet, orthopyroxene and plagioclase exsolution occur within clinopyroxene. There is no major- or trace-element compositional difference between garnet and clinopyroxenes included in graphite octahedra and those form-

ing the surrounding host rock (trace elements analysed by N. Shimizu, personal communication). This evidence implies that the inclusion and host-rock minerals have experienced the same pressure and temperature history, suggesting that silicate and diamond crystallization was co-genetic. This conclusion is further supported by the faceted nature of some inclusions.

Obducted ophiolites in Tibet<sup>22</sup> and Alpine peridotites in the Koryuk Mountains of the eastern Soviet Union and Armenia<sup>23</sup> are reported to contain very low concentrations of diamond. Alluvial diamonds, as yet of unexplained provenance but situated in the region of ophiolites or similar ultramafic belts including the Urals<sup>23</sup>, the Appalachians, Kalimantan (Borneo) and Copeton-Bingara (New South Wales), have no known volcanic sources<sup>24</sup>. The source rocks of these 'anomalous diamond occurrences', whether proven or suspected, are not eclogites but are mainly highly magnesian chromite harzburgites. These are similar to diamondiferous peridotite xenoliths in kimberlites except that garnet is usually lacking. Thus, rocks comparable to diamondiferous eclogite and peridotite xenoliths in kimberlite occur in some of the Earth's major collision zones, having been tectonically emplaced from close to the base of the lithosphere. Collision zones containing ultramafic massifs thus provide a potential economic source of diamonds.

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# Coagulation on bubbles allows microbial respiration of oceanic dissolved organic carbon

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DISSOLVED organic carbon in sea water (DOC) is one of the chief reservoirs of reactive organic carbon on the planet<sup>1</sup>. To determine the rate at which this carbon breaks down, a long-standing paradox must be solved. DOC appears to be remarkably unreactive<sup>2</sup>, yet it must be reactive to maintain the mass balance between organic carbon in the ocean and CO<sub>2</sub> in the atmosphere<sup>1</sup>. Here we show that the coagulation of colloidal DOC on bubble surfaces initiates the rapid microbial respiration of carbon which would otherwise be less accessible to the biota. This coupling of respiration to surface coagulation as a physical means of regenerating a substantial fraction (5–15%) of oceanic DOC could be a key factor in the mechanism required to recycle a recalcitrant reservoir of carbon back to CO<sub>2</sub>.

During August and September 1988, sea water was collected from a depth of 10 m on a transect of four stations across a highly productive tidal front on Georges Bank, and from four depths (from 10–85 m) at a station occupied for 9 days in the Sargasso Sea. On board ship all the sea water samples were filtered through 2- $\mu$ m Nuclepore membranes to remove the majority of particles without removing colloidal organic material<sup>3,4,5</sup> and were bubbled for 30 min in a glass column, using a 10–25  $\mu$ m glass frit to produce bubbles with an average diameter of 487  $\mu$ m (ref. 5). The air supplied to the frit was prefiltered through activated carbon and a sterile 0.2- $\mu$ m filter cartridge (Gelman) to ensure that contaminants were not introduced on bubble surfaces. After 30 min of bubbling, the sea water was divided into duplicate two-litre batches which were gently stirred in an incubator set at the appropriate *in situ* temperature. Two additional two-litre batches of filtered sea water were stirred at the same *in situ* temperature to act as unbubbled controls. Samples from all batches were taken at intervals to determine the rate of oxygen consumption by an oxygen gradient technique<sup>6</sup>.

The concept that aggregates of organic material and bacteria are generated by the coagulation of DOC on bubble surfaces has been discussed for over 25 years<sup>5,7</sup>. In our experiments this process of surface coagulation caused a rapid increase in microbial respiration, as measured by oxygen consumption rate (Figs 1 and 2). Respiration reached a maximum after 2–4 h and then declined to values similar to those found in unbubbled controls by 8–20 h. This is the first demonstration that surface coagulation can have a large, reproducible and systematic effect on microbial respiration in the open ocean—not only from the highly productive Georges Bank<sup>8</sup>, but also from a far less productive Sargasso Sea<sup>9</sup>. Without rapid sampling at the beginning of the experiments, we would have missed the dynamics associated with a transient microbial community and inferred that bubbling had no effect.

Many bubbling experiments have failed to show that surface coagulation has a significant effect on microbial communities<sup>5</sup>. Our experiments succeeded because we paid close attention to results obtained by Batoosingh *et al.*<sup>3</sup>, Johnson and Wangersky<sup>4</sup>, and Johnson *et al.*<sup>5</sup>. Filtering sea water through anything finer than a 2- $\mu$ m pore size removes larger colloids in the 0.2–1.2  $\mu$ m size range—a primary source of labile organic material for

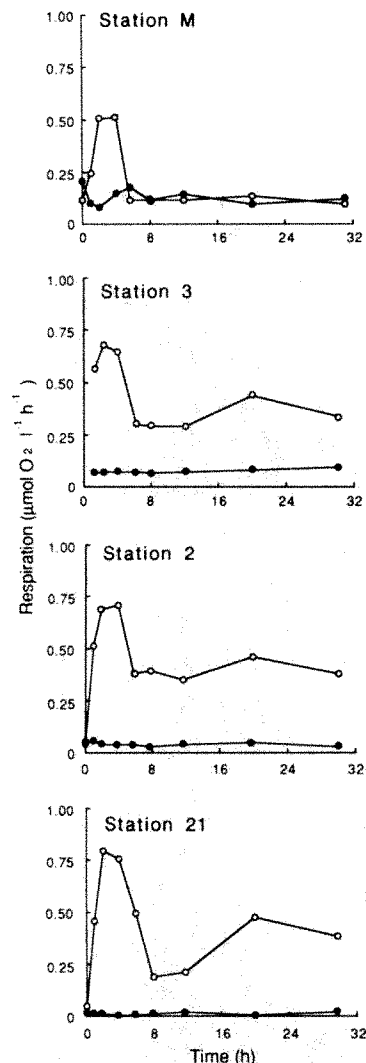


FIG. 1 Respiration in bubbled sea waters (open circles) and unbubbled controls (closed circles) from a depth of 10 m at four stations on the NE quadrant of Georges Bank (47° 00'N; 67° 51'W). The transect of 4 stations included surface-water environments ranging from the mixed layer of a stratified water column in the Fundian Channel (station 21) to a tidal front at the edge of the bank (stations 2 and 3) and finally to well-mixed, particle-rich water on the Bank itself (station M). Respiration was measured using an oxygen gradient technique<sup>6</sup>. Samples (10 ml) were pipetted into polycarbonate filter manifolds and filtered through 2- $\mu$ m Nuclepore filters. The same volume of filter-sterilized (0.2- $\mu$ m filtered) sea water was then added to each manifold and oxygen gradients were measured at *in situ* temperature to a distance of 5 mm above the material collected on the surface of each filter. The gradients allowed calculation of the downward flux of oxygen over a given length of time and thus the rate of consumption per cm<sup>2</sup> of surface or per ml of original sample. Error bars from selected triplicate incubations were all smaller than the dimensions of the symbols.

surface coagulation<sup>5</sup>. Physical models of the mass transfer of organic particles in turbulence<sup>10</sup> show that colloids of about 1  $\mu$ m in diameter are situated at the boundary between two domains of mass transfer. Particles smaller than 1  $\mu$ m are more rapidly transported to bacterial cells by convective diffusion, whereas larger particles are more effectively transported by collision with cells in turbulent shear. In effect, there appears to be an untapped reservoir of labile organic carbon that is not easily accessed by bacteria in the absence of surface coagulation. This in turn leads to an obvious but important conclusion. The interpretation of data from experiments designed to illustrate the microbial effects of bubbling will be seriously compromised if colloidal organic material has been removed by filtering.



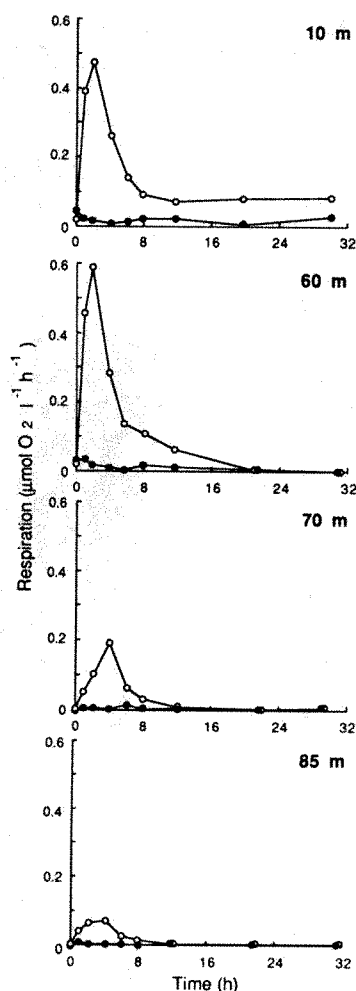


FIG. 2 Respiration in bubbled sea waters (open circles) and unbubbled controls (closed circles) from four depths at a station occupied for 9 days in the Sargasso Sea (at 36° 00'N; 64° 05'W). During this time, the mixed layer of surface water extended down to a depth of 60–70 m. Error bars from selected triplicate incubations were all smaller than the dimensions of the symbols.

On Georges Bank, the mean respiration in unbubbled controls increased (Fig. 1; Table 1) as the environment at a depth of 10 m progressed from the mixed layer of a stratified water column in the Fundian Channel (station 21) to a tidal front at the edge of the Bank (stations 2 and 3) and finally to well mixed, particle-rich water on the Bank itself (station M). Respiration was enhanced by surface coagulation to approximately the same absolute value at all 4 stations but, when compared with the controls, enhancement decreased by over an order of magnitude from station 21 to station M (Table 1). The reduced effect of surface coagulation in well mixed water at station M (ref. 11) was probably related to increased respiration of labile organics coagulated with larger numbers of suspended particles (1.1 p.p.m. at station M compared with 0.2 p.p.m. at station 21; P.E.K. and D.K. Muschenheim, unpublished observations). As a result, the effects of bubbling and surface coagulation may be of greater biological significance in sea water with low concentrations of suspended particulates, such as that found at station 21.

Bubbling enhanced respiration by a factor of 4.8–11.2 in the Sargasso Sea (Fig. 2; Table 1). This enhancement was positively correlated with primary production (Fig. 3) and decreased rapidly below the base of the mixed layer at 60–70 m (Fig. 2). Surface coagulation in this particle-poor environment may therefore be confined in its biological effect to relatively 'new' colloids associated with primary production in the upper ocean. The reasons why surface coagulation caused such a rapid microbial response are not clear, but the answer may lie in the results of Baylor *et al.*<sup>12</sup> and Graham *et al.*<sup>13</sup>, which show that bubbling sea water for 18–45 min can concentrate phosphorus into the adsorbed or coagulated phase by a factor of 4 to 200. Given that microbial growth is probably not carbon-limited in surface waters<sup>14</sup>, the degree to which nutrients such as phosphorus and nitrogen are involved in surface coagulation may ultimately regulate the magnitude of a microbial response to aggregate generation.

It still remains to be seen if bubble spectra in a glass column are typical of those found under a breaking wave, but it is our experience that data from bubbling columns may underestimate, rather than overestimate, the physical and biological effects of surface coagulation. A bubbling column can appreciably enhance the transport of 1 µm colloids to bacteria, but models of mass transfer of the same material by natural bubble populations under breaking waves<sup>10</sup> indicate that the enhancement may

TABLE 1 Mean respiration of bubbled sea waters and unbubbled controls in relation to previous measurements from Georges Bank and the Sargasso Sea

Location	Depth (m)	Respiration (unbubbled sea water) ( $\mu\text{g O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ )		Respiration (bubbled sea water) ( $\mu\text{g O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ )		Enhancement factor†
		Mean*	Range	Mean*	Range	
Georges Bank (this study)						
Station M	10	100	62–158 (n=9)	359	146–783 (n=9)	2.7
Station 3	10	58	54–73 (n=9)	369	223–523 (n=9)	6.4
Station 2	10	31	19–46 (n=9)	334	61–546 (n=9)	11.0
Station 21	10	9	3–15 (n=9)	323	68–510 (n=9)	35.8
Georges Bank <sup>21</sup>	surface	185	6–288 (n=6)	—	—	—
Georges Bank and Gulf of Maine <sup>22</sup>	1–10	132	3–190 (n=10)	—	—	—
Sargasso Sea (this study)						
	10	12	5–28 (n=9)	134	18–364 (n=9)	11.2
	60	15	5–34 (n=8)	129	3–450 (n=8)	8.6
	70	6	2–12 (n=9)	40	3–148 (n=9)	6.7
	85	4	2–9 (n=8)	19	2–53 (n=9)	4.8
Sargasso Sea <sup>23</sup>	surface	118	5–230 (n=13)	—	—	—
Sargasso Sea <sup>24</sup>	20–75	90	40–230 (n=4)	—	—	—
Bedford Basin (this study)	10	31	15–46 (n=8)	361	31–576 (n=8)	11.8

Respiration in the unbubbled controls fell within the range of values from earlier work and respiration in many of the bubbled sea waters exceeded the range of values from earlier work. The systematic variations in respiration that we observed in the presence or absence of bubbling may therefore have already been observed in earlier studies as apparently random variations. See ref. 20 for previous measurements.

\* Mean respiration in the bubbling experiments was calculated from 8 or 9 oxygen consumption rates measured over a 32-h period.

† The enhancement of respiration was calculated from mean respiration (bubbled) divided by the mean respiration from an equivalent unbubbled control.

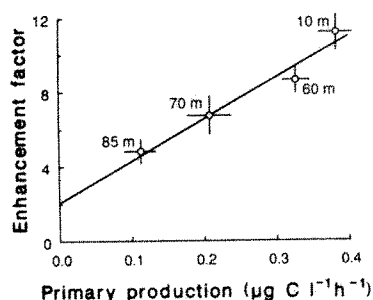


FIG. 3 Linear correlation of the enhancement of mean respiration by surface coagulation (Table 1) and *in situ* primary production at four depths in the Sargasso Sea (W. G. Harrison and B. Irwin, unpublished results). The equation of the line best fitting the data is  $y = 2.3 + 23.8x$  ( $r = 0.99$ ).

be too low by a factor of 2–20. This reinforces the concept that a substantial fraction of labile DOC may escape degradation by virtue of its particle size characteristics, and that surface coagulation is required to make the carbon available to bacteria.

The rapid respiratory responses in Figs 1 and 2 leave no doubt that the aggregates produced by coagulation are labile. When cumulative oxygen uptake was calculated from consumption rates on Georges Bank, 10–13  $\mu\text{mol O}_2 \text{ l}^{-1}$  were consumed over 32 h in bubbled sea waters from 10 m. This was equivalent to the respiration of 0.12–0.16  $\text{mg C l}^{-1}$ , or 10–15% of the 1.1  $\text{mg C l}^{-1}$  of DOC available for coagulation, as estimated from DOC analyses using the ultraviolet oxidation technique of Cauwet<sup>15</sup> (P.E.K., unpublished results). There is some uncertainty<sup>16,17</sup> and controversy<sup>18</sup> involved in the interpretation of DOC measurements in sea water, but the respiration of 10–15% of a given DOC pool in as little as 32 h indicates that significant quantities of carbon could be made available to the biota by surface coagulation. Even if the measurement controversy is resolved and there is more DOC than was previously thought in the upper ocean<sup>17</sup>, there is still a wide variation in the quantity of material coagulated by bubbling. Only 5% of the DOC pool (0.035  $\text{mg C l}^{-1}$  from a pool size of 0.7  $\text{mg C l}^{-1}$ ; P.E.K., unpublished results) was respired over 32 h when Sargasso sea water from 10 m was bubbled. Although this was 2–4 times lower than the carbon respired at the same depth on Georges Bank, primary production (4.5  $\mu\text{g C l}^{-1} \text{ d}^{-1}$  compared with 50–400  $\mu\text{g C l}^{-1} \text{ d}^{-1}$  on Georges Bank; W. G. Harrison and B. Irwin, unpublished results) was 12–88 times lower. This means that the physical regeneration of organic material by coagulation on bubble surfaces may have a more pronounced effect on microbial communities in regions of low productivity like the Sargasso Sea.

The results obtained when sea water from 10 m in Bedford Basin, Halifax Harbour (Canada) was bubbled during June 1988 (Table 1) demonstrate that respiration in a coastal environment can be enhanced by a factor within the range of values from the open ocean. More importantly, bacterial counts by epifluorescence<sup>19</sup> showed that a rapid increase in bacterial number (to values as high as  $16 \times 10^6 \text{ cells ml}^{-1}$ ) was associated with the respiration induced by surface coagulation. Bacterial growth was then followed by a decline as protozoan numbers increased, and the end result of 32 h of intense microbial activity was a relatively low number of bacteria ( $1.6 \times 10^6 \text{ cells ml}^{-1}$ ). Thus, when different amounts and different fractions of DOC are made available as different sea waters are bubbled, the rapidity of the biological response to surface coagulation is a common factor which cannot be ignored. The coagulation of DOC by bubbling may well be an important oceanographic process in its own right<sup>5,10</sup> but, when combined with rapid respiration of the coagulated material, it could also be a mechanism for recycling a globally important reservoir of carbon<sup>1</sup> back to  $\text{CO}_2$  in the upper ocean. □

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## Developmental failure and loss of reproductive capacity in the rare palaeoendemic shrub *Dedeckera eurekensis*

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EVOLUTIONARILY successful outcrossing plants have spontaneous seed sets that are, on average, around 50% ( $\pm 20\%$ ), but those of woody species are lower (33%) and herbaceous perennials higher (57%); self-pollinating (autogamous) species average about 85%<sup>1–3</sup>. *Dedeckera*, an outcrossing (protandrous) evolutionary relict from the Mojave desert of California has a seed set of only 2.5% and no means of vegetative reproduction. We show here that the most probable explanation for this exceptional result is that there are genetically mediated embryonic abnormalities. The percentage of fully viable seeds is further reduced by low viability, germinability, and post-germination developmental failure. Genetic studies involving extinction have emphasized inbreeding depression and homozygosity<sup>4–8</sup>, but *Dedeckera* is highly heterozygous (27%). Outcrossing does not significantly increase seed set over selfing, suggesting that a high segregational genetic load is primarily responsible for the low seed set in *Dedeckera*. Such loss of reproductive capacity could explain the relictual nature of *Dedeckera* and might ultimately result in its extinction.

A histological analysis of post-fertilization development of 27 ovules of *Dedeckera* showed a pattern of genetically controlled embryo abortion similar to that reported in *Epilobium*<sup>3</sup> (Onagraceae), and *Arabidopsis*<sup>9,10</sup> (Brassicaceae), and in species hybrids<sup>11</sup>. Three ovules showed no evidence of fertilization.

Spontaneous seed germination in seven trials was only 3.5% ( $N = 460$  filled seeds). Sixty days of stratification had no effect, but scarification and gibberellic acid treatment increased germination to 33%. Only 11.1% of spontaneously germinating seeds

‡ Deceased.

developed normally, but the sample size was small ( $N=9$ ). Thus the percentage of fully viable seeds in *Dedeckera* is probably less than 0.5%. *Dedeckera* may produce about 64,500 flowers and ovules (ovaries are uniovulate). Given seedling survivorship values for desert perennials<sup>12,13</sup> of  $10^{-4}$  to  $10^{-5}$ , the loss of reproductive capacity in *Dedeckera* may be critical for its continued survival.

Neither prezygotic genetic self-incompatibility nor inadequate pollination explain the exceptionally low fecundity of *Dedeckera* because about 90% of the ovaries initiate growth, which is taken as *prima facie* evidence for fertilization (Table 1). The consistency of the data, both between populations and in different years, indicates that resources do not limit seed set (Table 1), and also argues against gametic imbalance and hybrid dysgenesis as explanations for low seed set. *Dedeckera* is not a chromosome translocation heterozygote ( $n$  is about 12 bivalents). Herbivory was uncommon and there was no overt evidence of pathogen activity.

Intraplant self-pollination (geitonogamy) may be common in *Dedeckera*<sup>14</sup>, but inbreeding depression resulting from selfing in a normally outcrossed population (segregational genetic load) is not a major factor contributing to the low seed set (Table 2). The seed set for controlled self-pollinations (6.3%) and cross-pollinations (11.4%) do not differ significantly ( $d.f.=1$ ,  $\chi^2=2.95$ ,  $P>0.05$ ) and all population sizes exceed 150 (Table 2).

Allozyme analyses were completed from each of the six populations studied (Table 1) to determine the amount of genetic polymorphism and heterozygosity.  $F$ -statistics<sup>15</sup> were used to examine apportionment of variation within and between populations. Mean values, summed for all populations, are  $F_{IS}=0.192$ ,  $F_{IT}=0.299$  and  $F_{ST}=0.133$ , which indicate that there is a limited degree of interpopulational differentiation with most variance residing within populations.

Polymorphism over 17 loci and six populations is 56.8%. Heterozygosity averaged over the subset of the 12 polymorphic loci is 26.9% (Table 3). Heterozygotes range from 11.9% for

TABLE 1 Seed sets in *Dedeckera eurekaensis*

Population*	Collection year	% Developed (filled)	% Developed (aborted)	% Partially developed	% Non-developed	$n$ (ovaries)	$n$ (plants)
Bishop†	1988	1.6	6.5	39.9	52.0	1,624	10
	1987	0.6	20.2	72.5	5.8	1,046	10
	1984	4.6	50.1	24.5	20.8	351	1
Coldwater Cyn‡	1988	4.7	25.8	67.4	2.0	2,875	10
	1987	4.2	48.7	44.3	2.8	1,112	10
	1985	1.9	33.3	44.8	20.0	105	1
Gunter Cyn§	1988	0.8	41.3	32.9	25.0	1,482	10
	1987	1.9	54.1	36.2	7.8	1,238	10
Dedeckera Cyn	1988 (upper)	4.0	29.0	65.1	1.7	1,682	10
	1987	1.7	67.3	28.5	2.4	1,230	10
	1988 (lower)¶	4.3	44.3	49.6	1.8	1,464	10
	1987	2.9	61.3	33.2	2.6	1,265	11
	1978	1.7	46.0	42.5	9.7	113	1
	1975	2.9	29.7	50.5	16.8	101	1
Last Chance Mts#	1987	1.9	70.4	26.7	1.0	1,184	10
Panamint Mts**	1980	0.9	49.5	26.6	22.9	109	1
Means or totals (overall)		2.5	42.3	42.8	12.2	16,981	116
Means or totals	1988	3.1	29.4	51.0	16.5	9,127	50
Means or totals	1987	2.2	54.6	39.5	3.7	7,075	61
Means or totals	1975-85	2.4	44.5	33.5	18.9	779	5

The structures scored are anatomically indehiscent, single-seeded, post-anthesis ovaries or maturing fruits (achenes), although functionally they behave essentially as seeds. Developed (aborted) fruits reach approximately full length, but the ovary wall is partially collapsed between the ribs and the ovule has atrophied. Partially developed fruits did not reach full size, but all initiated ovary expansion before ovule abortion, which is taken as *prima facie* evidence for fertilization. Non-developed ovaries showed no evidence of expansion. They were probably not pollinated, or the ovules were unfertilized, or abortion occurred at the earliest stages of embryogenesis or endosperm development.

\* All locations on Universal Transverse Mercator grid ticks, zone 11; Cyn, Canyon; Mts, mountains. † 385500E-4136800N; ‡ 384000E-4149000N; § 388100E-4146200N; || 443700E-4101900N; ¶ 443600E-4101300N; # 442000E-4094800N; \*\* 434400E-4083100N.

TABLE 2 Controlled pollinations in *Dedeckera eurekaensis*

Pollination treatment	Per cent seed types recovered				$N$ 1-ovuled (ovaries)	$N$ (plants)
	Developed (filled)	Developed (aborted)	Partially developed	Non-developed		
Intrapopulation crosses	11.4	21.1	66.7	0.88	228	10
Self-pollinations (geitonogamous)	6.3	7.9	85.7	0	63	4
Interpopulation crosses ( $\times$ Gunter Cyn.)	12.9	37.1	48.4	1.6	62	2
Spontaneous seed set in parental plants	4.7	25.8	67.4	2.0	2,875	10
Flowers isolated in nylon enclosures	3.9	26.0	65.5	4.6	281	3

Coldwater Canyon population, 2-29 July 1988 (see Table 1 legend for locality and explanation of fruit types). Control flowers were taken from unmanipulated portions of experimental plants and left open so that pollination was spontaneous. Isolated flowers were those simply left under nylon enclosures (after removing all open or fruiting flowers), thus excluding all insect pollen vectors. All open or fruiting flowers were removed from the experimental inflorescences after which they were enclosed with removable fine-mesh nylon covers mounted over a wire frame to prevent contact of the nylon with the flowers. Flowers were emasculated daily before anthesis. All three stigmas were pollinated by contacting their sticky surfaces with a dehiscing anther after the styles had diverged and the stigmas became receptive. The small flowers (2-3 mm) required that all floral manipulations be effected under a dissecting microscope mounted on a camera tripod. The nylon covers were removed after the completion of all pollinations.



TABLE 3 Genetic variability at 12 loci

	Mean sample size per locus	Mean no. of alleles per locus	% Loci polymorphic	Mean heterozygosity
White Mountains populations				
Bishop	15.8	1.9	66.7	0.211
Coldwater Cyn	18.8	2.6	75.0	0.218
Gunter Cyn	16.3	2.3	75.0	0.194
Means	17.0	2.3	72.2	0.207
Last Chance Mountains populations				
Dedeckera Cyn (upper)	14.8	2.7	91.7	0.384
Dedeckera Cyn (lower)	16.7	3.3	91.7	0.293
Last Chance Mts	13.3	2.7	83.3	0.318
Means	14.9	2.9	88.9	0.332

Values in this table are derived from analysis of 12 loci—EST-2 (esterase), GSR-2 (glutathione reductase), IDH-2 (isocitrate dehydrogenase), MDH-1, MDH-2, MDH-4 (malate dehydrogenase), MDR-1 (menadione reductase), GPI-2 (glucose phosphate isomerase), PGM-1, PGM-2, PGM-3 (phosphoglucosmutase) and SKDH (shikimate dehydrogenase)—that were polymorphic in at least one of the six populations. When five additional loci ADK-1 (adenylate kinase), GPI-1, GSR-1, LAP (leucine amino peptidase), and MDH-3, monomorphic for all populations, are included, the mean polymorphism is 56.8%. A locus was considered polymorphic if any allelic variant was detected. Mean proportion of heterozygotes (direct count) is less than the expected Hardy-Weinberg (unbiased estimate)<sup>24</sup>. The fixation index  $F^{25}$  showed that only two loci (PGM-1 in the Gunter Cyn population and SKDH in the population of the Last Chance Mountains) had significant ( $P < 0.001$ ) deviations in genotype proportions.

MDR-1 to 58.3% for GPI-2. These heterozygosity averages are underestimated because they include the zero values for those markers that are not polymorphic in the particular population sampled. This amount of variation is much higher than that reported for other species of desert shrubs<sup>16</sup>, but is similar to other woody, outcrossing perennials<sup>17-18</sup>.

*Dedeckera* is vegetatively vigorous and flowers profusely. Its longevity (at least 140 years and probably much more) may be correlated with heterozygosity as reported in *Liatris*<sup>19</sup> (Asteraceae). In *Dedeckera*, vegetative fitness may be largely dependent upon rare, highly heterotic (or possibly epistatic) genotypes. The uniquely heterozygous genotypes that do survive may, however, have low reproductive potential because of the excessively high segregational genetic load. Reproduction could be further compromised by the accumulation of recessive lethals in long-lived meristems<sup>20</sup> and chromosomal mutations<sup>21</sup>.

The persistence of palaeoendemics may depend on rare multiple-locus heterotic genotypes that could arise as a result of tracking some protracted secular environmental change, for example, increasing aridity. The pace and extent of such change could easily exhaust the species' additive genetic variance relevant to adapting to that change. Heterosis may be the only survival strategy available to such species, in spite of the negative reproductive consequences. Thus the 13% increase in mean heterozygosity for the populations of *Dedeckera* in the Last Chance Mountains, as opposed to those in the White Mountains (Table 3), may be important because the former localities are both hotter and drier. In fact, many palaeoendemics may be ecologically 'out of place', in that they may not possess many of the adaptations typical of plants occupying that habitat—thus *Dedeckera* flowers in mid-summer when desert perennials are typically dormant.

Rabbits<sup>22</sup> and humans<sup>23</sup> have genetically mediated spontaneous embryo abortion rates of about 50% and 70%, respectively. The loss of reproductive capacity stemming from early embryonic genetic load should not be overlooked as a possible element in the decline of higher animal palaeoendemics. Such cases may be observed rarely because higher animals generally have much shorter life-spans, relatively limited reproductive potentials, and because early abortion is more difficult to detect than in plants.

Other palaeoendemic plants in North America, Africa and Australia also seem to have exceedingly low seed sets. Reproductive capacity should be given careful consideration in management decisions regarding rare and/or endangered species. □

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## The *ninaA* gene required for visual transduction in *Drosophila* encodes a homologue of cyclosporin A-binding protein

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MUTATIONS of the *Drosophila melanogaster ninaA* gene affect phototransduction: *ninaA* mutant flies have a 10-fold reduction in the levels of rhodopsin in the R1–R6 photoreceptor cells<sup>1,2</sup>. The *ninaA* gene was isolated and found to encode a 237-amino-acid protein that has over 40% amino-acid sequence identity with the vertebrate cyclosporin A-binding protein, cyclophilin, a protein that seems to be involved in T-lymphocyte activation. The remarkable evolutionary conservation of cyclophilin in two phylogenetically distant organisms and its involvement in diverse transduction processes suggests that this protein plays an important role in cellular metabolism. Indeed, cyclophilin has recently been shown to be a prolyl *cis-trans* isomerase that catalyses, *in vitro*, rate-limiting steps in the folding of a number of proteins<sup>3,23</sup>. Here, we present evidence for the involvement of cyclophilin-like molecules in a defined cellular process. The availability of mutations in a cyclophilin gene provides a new model system for the study of cyclophilin and cyclosporin action.

Numerous mutations that affect visual transduction in *Drosophila* have been isolated (reviewed in refs 4, 5). To isolate genes encoding phototransduction-specific proteins, we used a subtractive hybridization protocol to identify genomic clones

containing sequences preferentially expressed in the adult visual system. Clones that tested positive in our screen (see Fig. 2 legend) were isolated and used in *in situ* hybridizations to polytene chromosomes to determine their cytogenetic map positions. The *ninaA* locus has been mapped to the second chromosome, position 21D3-E2 (ref. 1). Two of the 120 genomic clones we isolated,  $\lambda$ 322 and  $\lambda$ N4, were mapped to position 21D4-E2, within the cytogenetic map location of *ninaA*. The *ninaA* locus constitutes one of eight complementation groups of *Drosophila* mutants with drastically reduced rhodopsin levels<sup>1,2</sup>. *ninaA* flies have a severe reduction of rhodopsin (Rh1) levels in the R1-R6 photoreceptor cells (Fig. 1). The reduction of rhodopsin levels in the R1-R6 photoreceptors of *ninaA* mutant flies has recently been shown<sup>6</sup> not to be due to reduced expression of the R1-R6 opsin gene (*ninaE*) but may be the result of a defect in some aspect of post-translational processing of the *ninaE* opsin, or a secondary effect on the stability of rhodopsin due to a defect in phototransduction.

Characterization of clones  $\lambda$ 322 and  $\lambda$ N4 showed that they contain overlapping sequences and encode a 0.9 kilobase (kb) RNA (Fig. 2) that is present in preparations of poly (A)<sup>+</sup> RNA from wild-type heads, but not from wild-type bodies or heads from mutant flies lacking the compound eyes (*eya*; eyes absent<sup>7</sup>) (Fig. 2b). We used the large *Eco*RI restriction fragment of  $\lambda$ 322 to screen a *Drosophila* head cDNA library and isolated several cDNA clones. Using M13 dideoxynucleoside triphosphate sequencing we have determined the DNA sequence of one of those cDNA clones and of the 1.2 kb *Hind*III-*Pst*I genomic fragment of  $\lambda$ 322 (Fig. 2a). Figure 3a shows the nucleotide sequence and the deduced amino-acid sequence of the *ninaA* gene product. The structure of the RNA (Fig. 2a) was determined by analysing genomic and cDNA sequences. Confirmation that  $\lambda$ 322 includes the *ninaA* gene was obtained by determining the nucleotide sequence of the *ninaA*<sup>P228</sup> mutant allele. Transcript size and levels are normal in these mutant flies (Fig. 2b). The mutant allele was isolated and cloned by a polymerase chain reaction. The *ninaA*<sup>P228</sup> allele has all the nucleotide sequence polymorphisms of the Oregon-R strain. Interestingly, however, this allele has a single coding nucleotide change at position 895, from TGG to TGA, causing a change from the encoded tryptophan to an opal translation termination codon (Fig. 3a arrow). The nature of this change is consistent with the chemical mutagenic origin of *ninaA*<sup>P228</sup> (ref. 1). Thus, the cytogenetic location, expression profile, and altered nucleotide sequence in mutant flies are all consistent with this being the *ninaA* gene.

Comparison of the deduced amino-acid sequence of *ninaA* with previously sequenced genes and proteins revealed very high homology to the bovine cyclosporin A-binding protein cyclophilin (Fig. 3b). These two proteins have more than 40% amino-acid identity throughout their length (shaded boxes); conserved

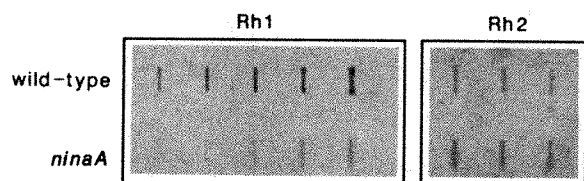


FIG. 1 *ninaA* mutants have a severe reduction of Rh1 opsin levels. Protein extracts were prepared from the heads of control and *ninaA* flies by digitonin extraction<sup>23</sup>. Serial dilutions (0.25, 0.5, 1, 2 and 4  $\mu$ g) were slot-blotted onto nitrocellulose paper and processed for western analysis using a monoclonal antibody against the Rh1 opsin (kindly provided by Dr H. Gert de Couet). Mutant flies have a 8–10-fold reduction of the opsin present in the R1–R6 photoreceptors (Rh1). The right panel shows similar extracts (4, 2 and 1  $\mu$ g) treated with a monoclonal antibody against the ocellar (Rh2) opsin. This monoclonal antibody was generated against a Rh2-specific synthetic peptide. Note the specificity of the mutant phenotype for the Rh1 opsin (see also refs 1, 2).

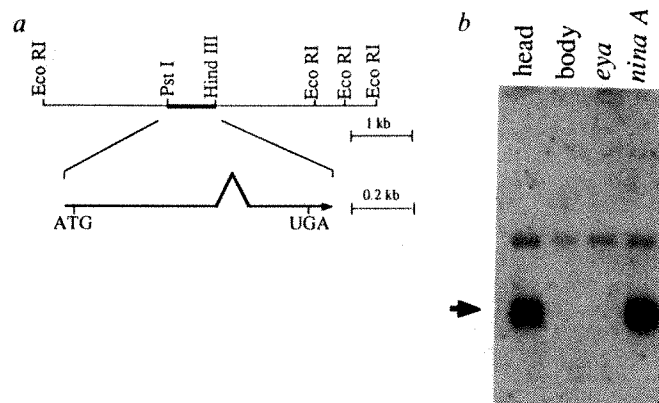


FIG. 2  $\lambda$ 322 encodes an eye-specific transcript. a, Restriction map of  $\lambda$ 322 and structure of the RNA it encodes. A map of  $\lambda$ 322 indicating the restriction sites for *Hind*III, *Pst*I and *Eco*RI (top map). The diagram below the map shows the structure of the RNA as deduced by comparison of the nucleotide sequence of a cDNA clone and the genomic sequence. b, Poly (A)<sup>+</sup> RNAs were extracted from adult heads of wild-type, *eya* and *ninaA* flies and from adult bodies. The RNAs (3  $\mu$ g per lane) were gel-fractionated, blotted, and hybridized to a 1.2-kb  $\lambda$ 322 radiolabelled *Pst*I-*Hind*III fragment.  $\lambda$ 322 hybridizes to a 0.9-kb eye-specific transcript (arrow). The blot was also hybridized to a probe encoding 'common' RNAs to control for the amount of RNA loaded ( $\lambda$ 9, unpublished observations). Hybridization to  $\lambda$ 9 mRNA is seen as the band present in all lanes with a mobility significantly slower than  $\lambda$ 322 mRNA. An RNA ladder (BRL) was used to provide size markers.

METHODS.  $\lambda$ 322 was isolated by hybridization with eye-specific probes. Poly (A)<sup>+</sup> RNA, isolated from the heads of wild-type flies, was used to template the synthesis of cDNA. This cDNA was hybridized in solution to a 20-fold excess of poly(A)<sup>+</sup> RNA isolated from the bodies of adult flies. Single-stranded molecules were then separated from the double-stranded DNA:RNA heteroduplexes by hydroxylapatite chromatography<sup>24</sup>. Hybridizations were carried out in 30  $\mu$ l of 0.5 M phosphate buffer (pH 6.9) containing 2  $\mu$ g of head cDNA and 40  $\mu$ g of body RNA. The reaction mixtures were incubated for 24 h at 65 °C. The resulting single-stranded cDNA, representing mostly 'head-specific' sequences, were used in a second round of subtraction with RNA isolated from heads of flies carrying the *eya* mutation<sup>7</sup>. Single-stranded molecules were fractionated by hydroxylapatite chromatography and used as templates for the synthesis of very high specific activity second-strand cDNA by two rounds of amplification with random primers (pN<sub>6</sub>, Amersham). This radiolabelled cDNA, representing a probe highly enriched in eye-specific sequences, was then used to screen a genomic library. Subtractions with *eya* head poly(A)<sup>+</sup> RNA were carried out with 0.2  $\mu$ g of head-enriched cDNA and 4  $\mu$ g of *eya* RNA. About 10% of input cDNA was recovered after the first subtraction, and about 20% after the second. First and second-strand cDNA synthesis were carried out as described<sup>25</sup>.

substitutions account for over 30% of the remaining amino-acid residues. Cyclophilin is a soluble protein with a relative molecular mass ( $M_r$ ) of 17,000 with high binding affinity and stereospecificity for cyclosporin A (CsA)<sup>8–11</sup>. CsA is an 11-amino-acid cyclic peptide of fungal origin with potent immunosuppressive properties widely used to prevent graft rejection and for the treatment of autoimmune disorders (reviewed in refs 12–14). Recent data support the hypothesis that the action of CsA is mediated through cyclophilin<sup>15–17</sup>. Although the exact mechanism of CsA action is not known, there is good evidence from whole cell systems that CsA interrupts an early event in the antigenic activation of T helper cells that results in lymphokine production<sup>18–20</sup>.

To determine whether *Drosophila* does indeed contain proteins with CsA-binding activity, we carried out [<sup>3</sup>H]CsA binding assays on head extracts prepared from wild-type controls, *eya*, and *ninaA*<sup>P228</sup> flies. As shown in Fig. 4, head extracts have significant levels of cyclophilin-like activity (specific partition on sepharose LH-20 chromatography<sup>8–11</sup>); about 30% of the head binding is associated with the visual system in that it is removed by the *eya* mutation. The *ninaA*<sup>P228</sup> mutation leads to a similar decrease in CsA binding indicating that the *ninaA*

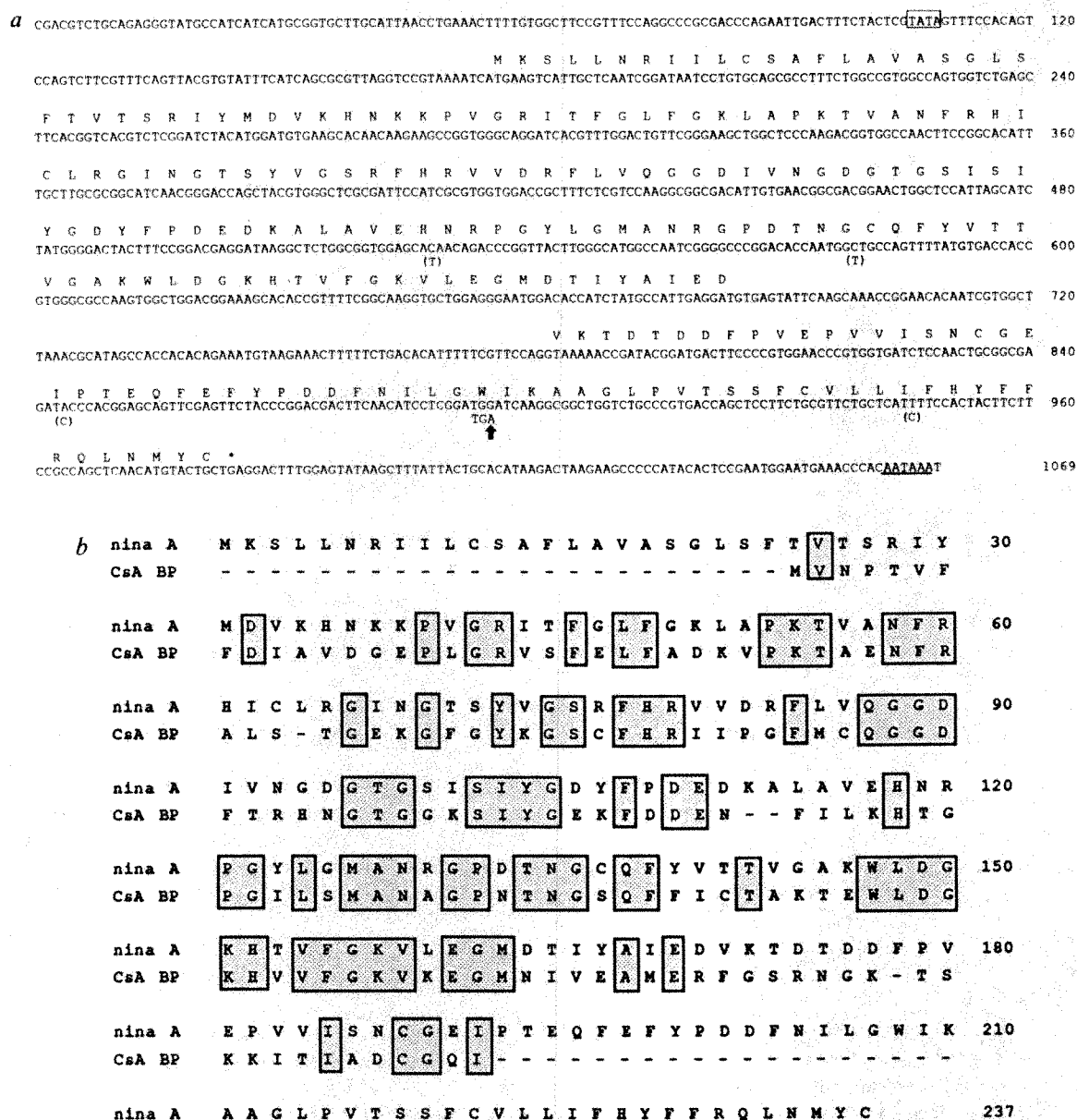


FIG. 3 Nucleotide sequence and deduced amino-acid sequence of the *ninaA* gene. **a**, The sequence shown was determined on both strands of the genomic and cDNA clones<sup>26</sup>. The boxed region at positions 106–110 shows the putative TATA box. The putative polyadenylation signal is underlined at positions 1,063–1,068. The deduced protein sequence is shown aligned under the nucleotide sequence. The initiator methionine was assigned as the first in-frame methionine in the sequence. The gap at nucleotides 686–782 indicate the presence of a 96-base pair (bp) intron. The arrow at position 895 highlights the single nucleotide change in the *ninaA*<sup>P228</sup> mutant gene (G→A). This change creates a translation termination codon. The single nucleotides below positions 528, 579, 844 and 946 show the differences in the coding regions between the cDNA (Oregon R, P2 strain) and the genomic (Canton S) sequence; none of these polymorphisms would result in a change in the encoded amino

acid. **b**, A co-linear alignment of the deduced amino-acid sequence of the *ninaA* gene and the bovine cyclophilin protein<sup>10</sup>. Cyclophilin from mammalian species analysed so far have over 95% sequence identity, and the physical properties of the CsA binding proteins from other vertebrate species are remarkably similar<sup>10,27,28</sup>. Amino acids are designated by the single-letter code. The alignment has been optimized for the largest number of identities with the least number of gaps. Boxed areas indicate the amino-acid identities between the two proteins.

**METHODS.** The mutant allele was cloned by selective amplification of the *ninaA* gene by polymerase chain reaction using protocol and reagents supplied by Perkin-Elmer Inc. (PCR kit N801-0043). Oligonucleotides complementary to positions –8 to +11 and +986 to +1,005 were used as primers.

locus encodes a cyclophilin-like protein that accounts for most of the visual system-specific CsA-binding activity (Fig. 4). The binding activity remaining in the *eya* and *ninaA* extracts probably represents the presence of non-eye-specific cyclophilin-like proteins.

The *ninaA* R1–R6 photoreceptors have about 10% of wild-type levels of rhodopsin<sup>1,2</sup>. Zuker and co-workers<sup>6</sup> have previously shown that the Rh1 opsin gene is expressed at normal levels in *ninaA* mutants. The reduction of rhodopsin must therefore be a post-transcriptional or a post-translational event. The discovery that cyclophilin encodes a prolyl *cis-trans* isomerase<sup>3</sup>

suggests that it may be required for the correct folding of proline-containing polypeptides. We propose that this isomerase activity is necessary for the correct folding and the stability of rhodopsin in *Drosophila* R1–R6 photoreceptors. The requirement for large amounts of rhodopsin in these cells may explain the existence of a photoreceptor cell-specific form of this enzyme. Moreover, the existence of a cell-specific isoform suggests that there are specific substrates for these proteins and explains the presumed diversity of cyclophilin-like molecules (M.A.S. and C.S.Z., unpublished observations and refs 3, 10). It is therefore possible that, in the immune system, CsA blocks



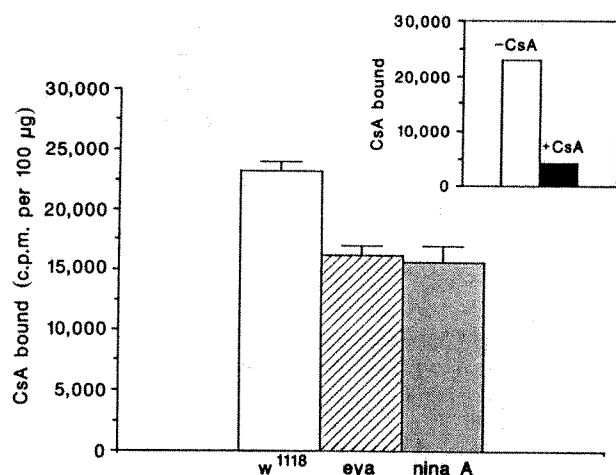


FIG. 4 *ninaA* flies have reduced levels of cyclosporin A-binding activity. Extracts prepared from heads of control *w*<sup>1118</sup> flies, *eya* or *ninaA* flies were tested for CsA-binding activity<sup>9</sup>. The results indicate that *Drosophila* head extracts contain significant amounts of cyclophilin-like activity (approximately 30 ng CsA binding mg<sup>-1</sup> extract) and that all eye-associated binding is reduced in *ninaA* flies (compare *eya* and *ninaA* extracts). Bars above the graph indicate standard errors (*w*<sup>1118</sup>, *n*=10; *eya*, *n*=8; *ninaA*<sup>P228</sup>, *n*=7). *Drosophila* extracts were prepared from heads of wild-type or mutant individuals exactly as described<sup>9</sup>. Binding assays were carried out with <sup>3</sup>H-cyclosporin A (17 Ci mmol<sup>-1</sup> Amersham) at either 30 °C or 37 °C and the products separated by partition chromatography on a Sephadex LH-20 column<sup>8</sup>. The inset shows the specificity of the binding assay as determined by competition with unlabelled CsA.

the activity of a cyclophilin required for the proper functioning of the antigen-mediated transduction pathway.

In the visual system, the interconversion between the active (metarhodopsin) and inactive (rhodopsin) states of the visual pigment molecule involves significant conformational changes. In the invertebrate visual cascade, these two forms are thermally stable and photoconvertible. It would therefore be very interesting to determine whether the isomerase encoded by *ninaA* is important in this event during the transduction cycle. The availability of *Drosophila* lines carrying mutations in the endogenous *ninaA* gene, and the use of P-element-mediated germline transformations<sup>21,22</sup>, may allow for the functional expression of wild-type and modified alleles in their normal cellular and organismal environment. A combined biochemical, physiological and molecular genetic dissection will help assign specific roles to the *ninaA* gene product in the phototransduction process. □

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## Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (*rds*)

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MUTANT mice homozygous for 'retinal degeneration slow' (*rds/rds*) are characterized phenotypically by abnormal development of photoreceptor outer segments in the retina, followed by gradual degeneration of photoreceptors<sup>1–3</sup>. This process of degeneration is complete by one year, with preservation of all other retinal cells<sup>4</sup>. The biochemical defect that leads to the mutant phenotype is not known. Our strategy for cloning the *rds* gene was based upon three previously reported observations. First, the *rds* locus maps to chromosome 17<sup>5,6</sup>. Second, experimental *rds/rds* ↔ +/+ and *rds*/+ ↔ +/+ tetra-parental mice manifest patchy photoreceptor changes in the retina<sup>7,8</sup>, suggesting that the wild-type *rds* locus is expressed within cells of the photoreceptor lineage. Finally, the process of degeneration is specific to photoreceptors. On the basis of these observations, we predicted that the *rds* mRNA is encoded by a gene on chromosome 17 and is normally expressed exclusively within photoreceptors in the retina. We here present evidence that this is the case.

Given our predictions, a cDNA representing a photoreceptor-specific mRNA encoded by a gene on chromosome 17 would be a candidate clone of the *rds* mRNA. To isolate cDNA clones of photoreceptor-specific mRNAs, we took advantage of the unrelated mouse mutant, retinal degeneration (*rd/rd*)<sup>9,10</sup>. Mice homozygous for this mutation manifest rapid degeneration of photoreceptors, a process that is virtually complete by four weeks, with the preservation of all other retinal cell types<sup>11,12</sup>. Therefore, an mRNA present in wild-type (C57BL/6) but absent from fully degenerate *rd/rd* (C3H/HeJ) retina is photoreceptor-specific. cDNA clones of twelve different photoreceptor-specific mRNAs were isolated from an adult C57BL/6 mouse retina library by subtractive and differential colony screening<sup>13</sup> of 6–7-week-old C57BL/6 minus 6–7-week-old C3H/HeJ retina. Northern blot hybridization patterns for six of these clones are shown in Fig. 1a.

The chromosome assignments for the genes encoding each of the 12 photoreceptor-specific mRNAs were made by probing a panel of mouse × hamster hybrid cell-line DNAs<sup>14</sup> with a representative clone of each of the photoreceptor-specific mRNAs. Clone IG3 mapped to chromosome 17 with 100% concordance

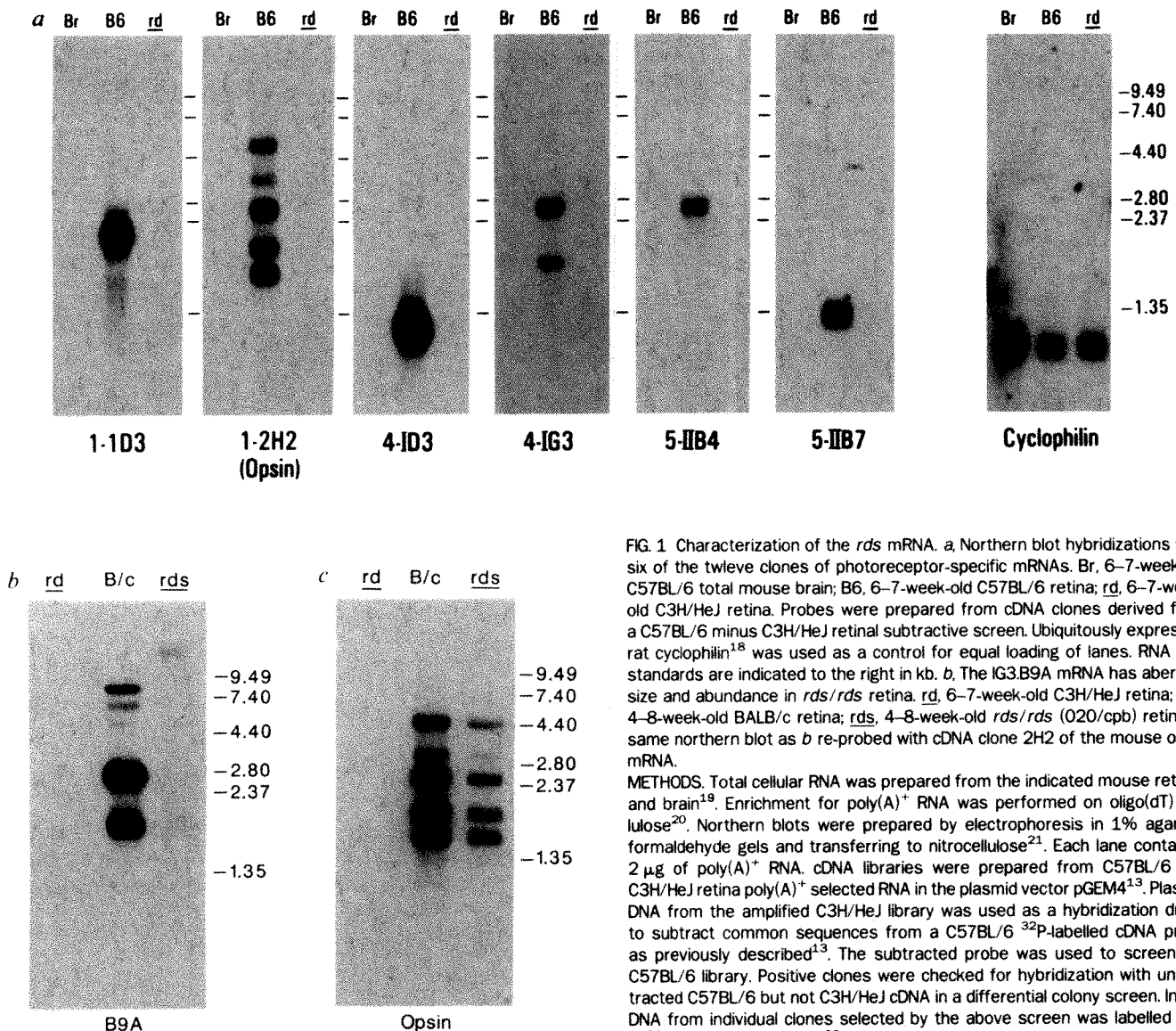


FIG. 1 Characterization of the *rds* mRNA. **a**, Northern blot hybridizations with six of the twelve clones of photoreceptor-specific mRNAs. Br, 6–7-week-old C57BL/6 total mouse brain; B6, 6–7-week-old C57BL/6 retina; rd, 6–7-week-old C3H/HeJ retina. Probes were prepared from cDNA clones derived from a C57BL/6 minus C3H/HeJ retinal subtractive screen. Ubiquitously expressed rat cyclophilin<sup>18</sup> was used as a control for equal loading of lanes. RNA size standards are indicated to the right in kb. **b**, The IG3.B9A mRNA has aberrant size and abundance in *rds/rds* retina. rd, 6–7-week-old C3H/HeJ retina; B/c, 4–8-week-old BALB/c retina; *rds*, 4–8-week-old *rds/rds* (O20/cpb) retina. **c**, same northern blot as **b** re-probed with cDNA clone 2H2 of the mouse opsin mRNA.

**METHODS.** Total cellular RNA was prepared from the indicated mouse retinas and brain<sup>19</sup>. Enrichment for poly(A)<sup>+</sup> RNA was performed on oligo(dT) cellulose<sup>20</sup>. Northern blots were prepared by electrophoresis in 1% agarose formaldehyde gels and transferring to nitrocellulose<sup>21</sup>. Each lane contained 2 µg of poly(A)<sup>+</sup> RNA. cDNA libraries were prepared from C57BL/6 and C3H/HeJ retina poly(A)<sup>+</sup> selected RNA in the plasmid vector pGEM4<sup>13</sup>. Plasmid DNA from the amplified C3H/HeJ library was used as a hybridization driver to subtract common sequences from a C57BL/6 <sup>32</sup>P-labelled cDNA probe as previously described<sup>13</sup>. The subtracted probe was used to screen the C57BL/6 library. Positive clones were checked for hybridization with unsubtracted C57BL/6 but not C3H/HeJ cDNA in a differential colony screen. Insert DNA from individual clones selected by the above screen was labelled with [<sup>32</sup>P]dCTP as described<sup>22</sup> and was used to probe northern blots. Final stringency wash conditions were 0.2 × SSC/0.2% SDS at 68 °C.

(Table 1). A full-length cDNA clone (IG3.B9A), containing an insert of approximately 2.7 kilobase (kb), was isolated by re-probing the C57BL/6 retinal cDNA library with clone IG3.

Clone IG3.B9A was used to probe northern blots containing RNA from the retinas of 1–2-month-old (pre-degenerate) *rds/rds* mice (BALB/c background) and age-matched wild-type (BALB/c) mice (Fig. 1b). Two major mRNA species of approximately 1.6 kb and 2.7 kb (estimated abundance, 0.05%) were observed in the BALB/c retina lane. These bands were not present in the *rds/rds* retina lane. A faint doublet band of approximately 12 kb was detected in the *rds/rds* lane however, which was not present in the BALB/c lane. Thus, the mRNAs detected with clone IG3.B9A in the retinas of *rds* mice are aberrant in size and abundance, suggesting that IG3.B9A is a clone of the wild-type *rds* mRNA.

When the same northern blot was re-probed with a cDNA clone of the opsin mRNA (clone 1-2H2 in Fig. 1a), the BALB/c and *rds/rds* retina lanes exhibited identical hybridization patterns, although the signal intensity in the mutant lane was slightly reduced (Fig. 1c), consistent with partial degeneration of photoreceptors. This experiment excluded the possibility that the observed difference in RNA hybridization patterns with the

IG3.B9A probe in wild-type and *rds/rds* retina was due to premature degeneration of photoreceptors in the mutant, or to a generalized abnormality in RNA processing within photoreceptors in *rds/rds*.

To define the site of the mutation within the putative *rds* locus, five adjacent non-overlapping restriction endonuclease fragments spanning the full-length cDNA clone IG3.B9A were prepared (Fig. 2a). These fragments were used individually as probes to examine restriction digests of genomic DNA prepared from BALB/c and *rds/rds* mice on Southern blots. Restriction fragment length polymorphisms (RFLPs) were detected in the DNA of BALB/c relative to *rds/rds* mice with probe B9A-II (Fig. 2b), but not with probes B9A-I, -III, -IV or -V (data not shown).

Genomic libraries prepared with the DNA from BALB/c and *rds/rds* mice were probed with cDNA clone IG3.B9A. A clone B7 was isolated from the BALB/c library and a clone R24 was isolated from the *rds/rds* library. The λ-cloned DNAs gave similar hybridization patterns to their corresponding wild-type or mutant genomic DNAs on Southern blots probed with cDNA clone fragment B9A-II (Fig. 2b).

To define the mutation at the nucleotide level, the 2.8 kb *Pst*I

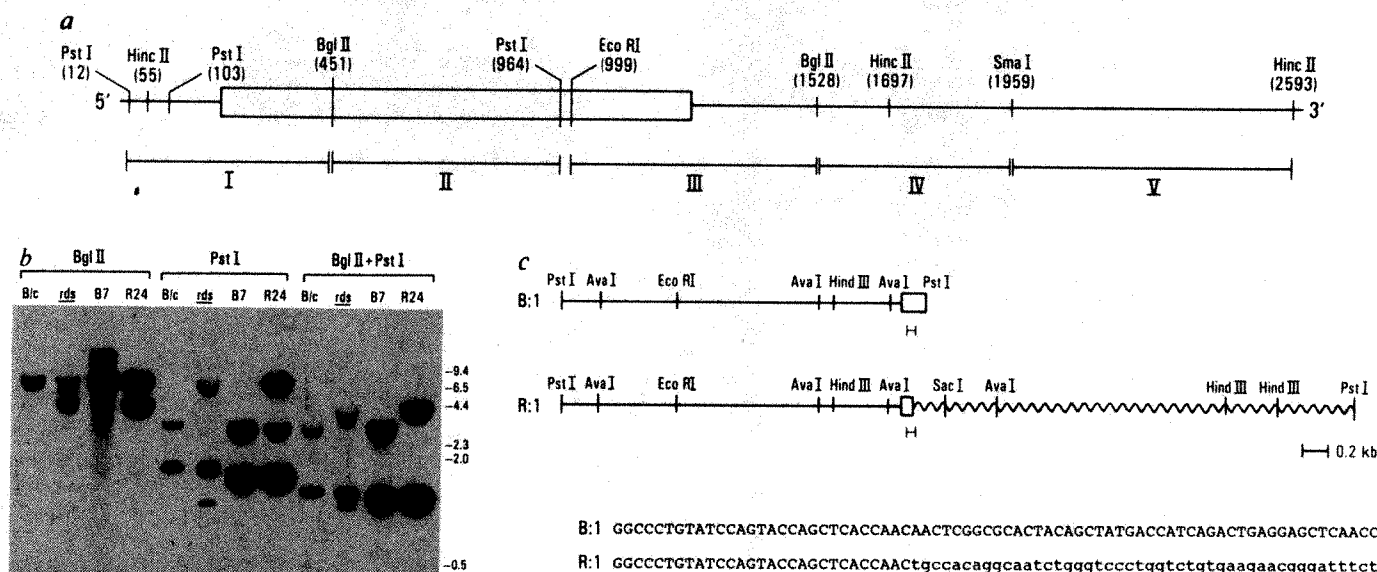


FIG. 2 **a**, Restriction map of the *rds* cDNA clone IG3.B9A derived from the nucleotide sequence (see Fig. 3). The open reading frame is indicated by the open box. Restriction fragments (B9A-I-V) used as probes for Southern blot analysis are indicated below. **b**, Southern blot analysis of genomic and  $\lambda$  clone DNA with IG3.B9A fragment II probe. B/c, BALB/c DNA; *rds*, *rds/rds* DNA (10  $\mu$ g genomic DNA per lane); B7,  $\lambda$  clone B7 from BALB/c library; R24,  $\lambda$  clone R24 from *rds/rds* library (0.5 ng  $\lambda$  clone DNA plus 10  $\mu$ g sheared salmon sperm DNA per lane). DNA samples were digested with restriction endonuclease *Bgl*II, *Pst*I, or *Bgl*II plus *Pst*I as indicated. DNA size standards are indicated to the right in kb. Electrophoresis, transfer to nitrocellulose, preparation of probes and stringency wash conditions as described in Table 1.

**c**, Restriction map of BALB/c and *rds/rds* genomic clones. B:1 is the 2.8 kb *Pst*I genomic subclone fragment from wild-type phage  $\lambda$  clone B7 detected with probe B9A-II. R:1 is the 6.3 kb *Pst*I genomic subclone fragment from *rds/rds* phage  $\lambda$  clone R24 detected with probe B9A-II. The open box indicates an exon which contains a protein-coding region (see Fig. 3). The wavy line indicates the insertion in the mutant locus. The nucleotide sequences of B:1 and R:1 in the region containing the divergence is indicated below. Capital letters in the nucleotide sequence correspond to the wild-type exon; lower-case letters correspond to the insertion. Bars underneath the restriction map show the origin of the indicated sequences.

TABLE 1 Segregation pattern of the mouse allele

Chromosome	Hybridization pattern				Per cent discordance
	+/+	-/-	+/-	-/+	
1	4	5	4	1	35.7
2	5	3	3	3	42.9
3	2	4	3	0	33.3
4	3	3	5	2	53.8
5	0	6	8	0	57.1
6	3	4	5	2	50.0
7	4	0	4	6	71.4
8	3	5	5	0	38.5
9	1	5	7	1	57.1
10	1	6	7	0	50.0
11	0	4	6	0	60.0
12	4	1	2	3	50.0
13	3	2	5	3	61.5
14	1	5	7	1	57.1
15	6	0	0	4	40.0
16	2	4	5	1	50.0
17	8	6	0	0	0.0
18	3	2	4	3	58.3
19	2	2	6	3	69.2
X	4	5	4	1	35.7

Southern blots containing DNA from mouse  $\times$  hamster somatic cell hybrids were probed with cDNA clone IG3. Column designations: +/+, chromosome and IG3 hybridization signal both present; -/-, chromosome and hybridization signal both absent; +/-, chromosome present and hybridization signal absent; -/+, chromosome absent and hybridization signal present. A hybridization signal corresponding to the hamster allele was present in all cell lines. 10  $\mu$ g DNA samples from 16 mouse  $\times$  hamster hybrid cell lines containing different mouse chromosomes<sup>14</sup> were digested with restriction endonuclease *Pst*I and resolved by electrophoresis on 0.7% agarose. The DNA was transferred to nitrocellulose<sup>17</sup> and then probed with DNA from clone IG3 (see Fig. 1). Final stringency wash conditions were 0.2  $\times$  SSC/0.2% SDS at 68  $^{\circ}$ C.

fragment from (wild-type)  $\lambda$  clone B7 and the 6.3 kb *Pst*I fragment from (*rds/rds*)  $\lambda$  clone R24 detected by B9A-II were both subcloned. Restriction endonuclease mapping of these subcloned *Pst*I fragments (B:1 and R:1) revealed that the 5' 2.7 kb of each were indistinguishable. After the third *Ava*I site (Fig. 2c), the restriction maps of B:1 and R:1 diverged. The 0.25 kb *Ava*I-*Pst*I 3' fragment from the wild-type subclone B:1, and the corresponding 0.95 kb *Ava*I-*Ava*I fragment from the mutant subclone R:1 were isolated and their nucleotide sequences determined. The sequences were identical up to the position corresponding to nucleotide 899 in the mRNA (see below a' Fig. 3). At this point, a foreign sequence appears in the mutant gene, disrupting the protein-coding exon (Fig. 2c). As the mutation occurs within an exon, this foreign sequence is probably transcribed in *rds/rds*. The observed increase in the size of the transcripts detected with clone IG3.B9A in the retinas of *rds/rds* mice is consistent with the insertion of approximately 10 kb of foreign DNA into an exon of the *rds* gene.

In an attempt to identify this foreign insert, we used Southern blots to analyse genomic DNA isolated from BALB/c and *rds/rds* using the 0.4 kb *Sac*I-*Ava*I restriction fragment from the inserted element of R:1 (Fig. 2c) as a probe. About 30 bands were detected in both the wild-type and *rds/rds* lanes, with two additional bands present in the *rds/rds* lanes that were absent from the wild-type lanes (data not shown). A computer database search was performed with the nucleotide sequence from the 0.4 kb *Sac*I-*Ava*I fragment of R:1 (data not shown). No significant similarity to any eukaryotic or viral sequences was detected. Based upon its size (approximately 10 kb) and its copy number in the mouse genome (10-30 copies), this inserted DNA may represent an unidentified retrovirus-like element.

Sequence analysis of the IG3.B9A cDNA revealed an insert containing 2,632 base pairs (bp) followed by a poly(A) tail (Fig. 3). A single long open reading frame began at nucleotide 213, encoding a putative protein of 346 amino acids. A common



**FIG. 3** Nucleotide sequence of the full-length *rds* cDNA clone IG3.B9A. Nucleotide numbering is shown on the left. The single long open reading frame has the corresponding amino-acid sequence translated above. The polyadenylation signal CATAAA for the 1.6 kb mRNA (IG3.D6), and ATTTAA for the 2.7 kb mRNA (IG3.B9A) are underlined. **METHODS.** The sequence was determined by a modified version<sup>23</sup> of the chemical degradation method of Maxam and Gilbert<sup>24</sup>. The complete sequence was determined for both DNA strands of clones IG3.B9A and IG3.D6 (see below). Primer extension analysis was performed on clone IG3.B9A as described<sup>25</sup> using a synthetic deoxyoligonucleotide corresponding to the antisense strand of IG3.B9A from position 64 to 41 (data not shown). cDNA clone D6 of the small, wild-type *rds* transcript was isolated by screening for clones that hybridized with full-length clone IG3.B9A but failed to hybridize to a truncated clone containing 0.6 kb from the 3' end of IG3. The sequence of clone IG3.D6 was identical to IG3.B9A except for the use of an alternative polyadenylation signal.

variant (AUUAAA)<sup>15</sup> of the consensus polyadenylation signal (AAUAAA)<sup>16</sup> was found 20 nucleotides upstream from the poly(A) tail. Primer extension analysis showed that IG3.B9A was only six nucleotides short of being a full-length clone of the 2.7 kb mRNA (see Fig. 3 legend). A cDNA clone of the 1.6 kb *rds* transcript (IG3.D6) was isolated by further screening (Fig. 3 legend). Sequence analysis of IG3.D6 showed that the 1.6 kb mRNA was identical to the 2.7 kb mRNA except that the poly(A) tail began 24 bases downstream from the alternative polyadenylation signal CAUAAA<sup>15</sup>, at position 1,651 rather than 2,633.

A computer search of a protein sequence database (Protein Identification Resource, 31 March 1988) with the predicted protein encoded by the *rd5* mRNA revealed no significant similarities to any known protein sequences. The *rd5* protein (relative molecular mass,  $M_r$  39,259) does not seem to have an N-terminal secretion signal sequence. It does contain three uncharged regions (amino acids 16-41, 100-122 and 252-275) which may represent membrane-spanning domains. Four sets of tandem basic residues (amino-acid residues: 11-13, 46-48, 178-179 and 324-325) are possible candidates for proteolytic cleavage signals. The protein also contains 13 cysteine residues which are potential sites for intra- or inter-chain disulphide linkages. As the *rd5* mRNA has been shown here to be photoreceptor-specific within the retina and is not detectable in brain, it is possible that the *rd5* protein function relates to some photoreceptor-specific process, possibly as an unidentified member of the visual transduction cascade. As the insertion occurred within a protein-coding exon, the resulting protein will be aberrant, lacking its normal C-terminal 87 amino-acid residues if translation of the aberrant *rd5* mRNA does occur in *rd5/rd5* retina.

From the evidence presented here, we concluded that we have cloned the *rds* gene and characterized its normal mRNA products. This is the first molecular description of a gene determining neuronal degeneration in a mammalian system where nothing was known in advance about the gene product. It remains a

90 AAGGACCTCTGCAGATACCGCGGGCTAGATTAGCTCCGGGTACCGTTACTGAGTTAAACGGGGATCCCAAGCTAGGAGAGGCCCAAAATGG  
GCAACTCCCTGCAGCTTSGGGCCATGGTGCTCTCTCCCTAGACACCTTAGCGGTCACGCCCGGAGCTACTCGGATTAGGATGGAAAGCTGA

MetAlaLeuLeuLysLysPheAspGlnLysArgValLysLeuAlaGlnGly

180 ACCGTGGGAGGGTCTGCTGAACGCACCTCGGTAAGCATGGGCTGCTCAAAGTCAAGTTTGACCAAGAAGCGGGTCAAGTTGGCCAGCGGG  
LeuTrpLeuMetAsnTrpLeuSerValLeuAlaGlyIleValLeuLysPheLeuLysLeuGluLeuArgSerSer  
CTCTGGCTTATGAAGTCGGCTGTCCGTTGGCCGGCATGCTCTCTTCAGCTTGGGGCTGTCTTCTGAAGATTGAACATTCGCAAGAGGAGC

270 GluValMetMetAsnSerGluSerHisPheValProAsnSerLeuLeuGlyValGlyValLeuSerCysValPheAsnSerLeuAlaGly  
GAAGTGTATGAATTTCTGAGAGCCACTTTGTGCCAACCTCTCATGAGGGTGGGGTCTGTCTCTGTCTTCAACTCTCTGGCTGGG

360 LysIleCysTyrAspAlaLeuAspProAlaLysTyrAlaLysTrpLysProTrpLeuLysProTyrLeuAlaValCysIlePhePheAsn  
AAGATCTGCTATGATGGCTGCACCGGCCAAGTCAGCCAAAGTGAAGCCGCTGCTGTAAGCCGCTACCTGGCTGTCTGCATCTTCTTTAAC

450 ValLeuLeuLeuAlaLeuLysCysPheLeuLysArgLysLeuLysLeuTrpLeuLysLeuGlyLeuLysAsnGlyMetLys  
GTCACTCTCTCTCTGGTGCTCTCTGCTGCTTTCTGTGGCGGGCTCCCTGGAGAGCACCTCGCTTACGGACTCAAGAATGGGATGAAG

540 TyrTyrArgSerTrpAspThrProGlyArgGlyPheMetLysLysLysThrLeuMetLeuGlnIleLysCysCysGlyAsnAsn  
TATTTATCGGATACGGCAACCCCGCGGGTCTCTCATGAAGAAAGACCATGCATGCTCCAGATTGAGTTTCAAGTCTGTGGGAAACAA

630 GlyPheArgAspTrpPheGluIleGlnTrpIleSerAsnArgTyrLeuAspPheSerSerLysGluValLysAspArgIleLysSerAsn  
GGCTCTCGGAGACTGGTTGAGATTCAGTGGATACGCAATCGCTACTGGACTCTCTCTCCAAAGGAGTCAAAGATCGCATCAAGAGCAAC

720 ValAspGlyArgTyrLeuLysAlaAspGlyValProPheSerCysAsnProSerSerProArgProCysIleGlnTrpGlnLeuTrpAsn  
GTGGATGGCGGCTACCTGTGGAGCGCGTCCCTTCAAGTCTGTGCAACCCAGCTCCCGCGGGCCCTGTATCCAGTACCAGCTACCAAC

810 AsnSerAlaHisTyrSerTyrAspHisGlnThrGluAlaLeuLysLeuTrpLeuArgGlyCysArgAlaAlaLeuLysAsnTyrTyrSer  
AACTCGGCGCATACAGCATATGACCTACAGTACGGAGTCAAACTCTCGCTCGGGGGTCAAGGGCGCTCTGCTGAATTACTACAGC

900 SerLeuMetAsnSerMetGlyValValThrLeuLysValTrpPheLysValSerIleThrAlaGlyLeuArgTyrLeuHisThrIleAla  
AGCCTCATGAATTCATGGGCGTGTGCACACTTCTCGTCTGGCTCTTTAGAGTGAGACTACTGCGGACCTCGCTACCTCCACACAGCG

990 LeuGluSerValSerAsnProGluAspProGluCysGluSerGluGlyTrpLeuLysGluLysSerValProGluThrTrpLysAlaPhe  
CTGGAGAGTGTCTTAAACCGGAGGACCCCGAGTGTAGAGTGAGGGCTGGCTGCTGGAGAAGAGGCTGGCCGAGACCTGGAAGCGCTTT

1080 LeuGluSerPheLysLysLysLysSerAsnGlnValGluAlaGlyLysAlaGlyProAlaProGluAlaGly  
CTGGAGAGCTTTAAGAAGCTGGGCACAGCAACATCAGGTGGAGGGTGAAGTGCAGACGAGGCGCGGCTCCAGAGGCTGGCTGTGGCTT  
1260 GGGGCTCTCCGCCCTCTCAACACTTAGTGACTCCGAGGACTGTGGATACCCCTTGTCTCAGCTGAAGATCAAATTTCCCGAAGAAG  
CTGCTCAACTACTGACTCTCTTTGATGTGGGCTTGAAGTTAGGGCTCTTAGGGCAGGTACAAACATTTGTGAACCGCTGCCCTCCAG  
1350 ATGTGAGTGACTGAACATGACGACGATGCGGCAGGATCGAACGCTACAGGACTCGGCAGCTCAAGGGCTGTGTCCAAAGTGTGAGTCCAG  
1440 TCTCTCATAGGTGACTGGCCACCAAGGCGCTCTCCCTCTCATGATGTCTGCTCTTTTAAAGTACAAAGTCTGCATGTCCAACTCAT  
1530 CACTTGAACATCAATGAACCAAGGTGAATAAAGAAATCTCAAGGCGCATGTGTTTGTCTTCAATGATAGGTTAAACAGGCTGTGTA  
1620 TCATTTCGCTATTATGTACAACTGGGGGAGGAACATGATATTTTAAAGATATGGAAGTCTCAAGAGGTACTCCACATGATGAATA  
1710 TTACCATGGCACAGGAGGTAAAACTTCTTCTTAGTAAATTCAGTGAAGTGTCCCATAGCTGCCCATTCCCAGCTCGAGTCAAGTCA  
1800 ATCTPACAGTGGCATGTGCCAGATCTCCCACTACCAAGTGTCTGGGTGAGCCACTCTGTAAGAGCGCGGCTGAGTTTGTCTGCTCTT  
1890 GTGGGAGATGTGTGTGATCAATTAGAATGTGCCACTTGGGGACAAGCGCTGCTCTGTGTGTCTCAGATGGCCACAACAGTCTTTG  
1980 TGAAGTCACTGTGTCAGGAGAGGCAAGACCGCTGGGAGCTCTGTGTTCTCCCAACAAAGACTATGGGAATGCTCTCGTATCCAGAA  
2070 GATCTTGAGATAGATGCTTTTCCGAGCTAGCGTGTGGAAAGTGTGTTTCTTGTGGAAAGCTGACGGAAACTCTCAGGGAAGACA  
2160 AAAATATGATCTGTGTGGACATGTGTGGGATGGACCAACAAAGGACTGTGGTGGCCATCTGAGACACAGGAGGAGCGGCTGTGTCCC  
2250 AAGGCGTCAGTCTGCTGGCCTGTGCTGTGGTCTTCAACTCTCTCAGGGGGAAGACCATAGAGATCTGCGCATCGGCCAACCAACGA  
2340 ATGGCCCCAATGTTGGTGTAGTATTCTGAGTCTCTCAGAGCAAGAAATGGCCAGATGCTGAGGCAGGCATCTGTGGCGGGAGCCCTGGG  
2430 GTCTCGCGCTCAACCTCTCAGGAGGGCTGAGACTATGGTGAAGAAATCACTAGACCCATTTCTAACTATGCTGCTCAACATCGTATTAA  
2520 GATATTATTAACATATATTGTATG(A)<sub>n</sub>

formal possibility that the gene identified here is not *rdx*: if loss of function of the identified gene product is not lethal to photoreceptors and if another gene on chromosome 17 which is also expressed in photoreceptors bears an independent second mutation. Final proof that the identified gene is indeed *rdx* will await correction of the phenotype by introducing DNA from the cloned wild-type allele into the *rdx/rdx* background in a transgenic experiment. Elucidation of the mechanism of photoreceptor degeneration in *rdx/rdx* will need studies of the *rdx* gene product. The first steps towards this involve immunocytochemical localization of the *rdx* protein within photoreceptors, and the identification of other proteins which may be physically associated. □

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# Does T-cell tolerance require a dedicated antigen-presenting cell?

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ALMOST 30 years ago Burnet proposed that the immune system maintained self-tolerance by deleting autoreactive lymphocytes<sup>1</sup>. Recently it has become clear that for T cells this step occurs in the thymus, where developing T cells first express their antigen-specific receptors<sup>2-4</sup>. Here a T-cell which encounters its antigen disappears—if it is not dead, it at least stops expressing its receptors. In the periphery by contrast, encounter with antigen leads to activation and proliferation of the responding T-cell. There are two possible explanations for this difference. Either the antigen-presenting cells in the thymus are different from those in the periphery and instead of producing positive signals they directly or indirectly kill the thymocytes<sup>5,6</sup>; or the T cells themselves are different, and like immature B cells, may die after encounter with antigen<sup>7,8</sup>. We tested the first possibility and found that dendritic cells from spleen, which are the most potent activators of mature T cells<sup>9</sup>, are also the most potent inactivators of young developing T cells. Thus it is not the antigen-presenting cell which determines whether a T-cell responds or dies, but the T-cell itself or its thymic environment.

To analyse the activity of various cell types, we used *in vitro* thymus organ cultures<sup>10,11</sup>. We cultured embryonic mouse thymus lobes from 14-day-old fetal B6 or (B6 × BALB/c) mice for 7 days. At day 14 of gestation these thymuses have no mature T cells<sup>12,13</sup>, but by day 21 the T cells have matured<sup>11</sup> and can be activated by mitogens<sup>11</sup> or in mixed-lymphocyte reactions<sup>14</sup>.

Figure 1 shows the result of two experiments designed to find out whether it was possible to tolerize cytotoxic T cells developing *in vitro*. Since it had been shown that proliferating T cells can be rendered specifically unresponsive if they are co-cultured with allogeneic adult<sup>11</sup> or fetal<sup>15</sup> thymus cells, we cultured the fetal thymuses in contact with a slice of irradiated adult allogeneic (SJL or CBA) thymus. Figure 1a and c shows that fetal T cells can develop into cytotoxic T lymphocytes (CTL) specific for allogeneic C3H.Q, CBA or SJL stimulators. Fig. 1b and d demonstrates that co-culture with adult thymic tissue generates specific unresponsiveness. Thymuses co-cultured with CBA (Fig. 1b) no longer generated CTL against CBA, but remained totally responsive to C3H.Q, whereas thymuses co-cultured with SJL became tolerant of SJL while maintaining their activity against CBA (Fig. 1d). Using limiting-dilution analysis (not shown), we found that the frequency of cells responding to the co-cultured thymus was decreased 100- to 1000-fold. Thus it was clear that the *in vitro* development of immature T cells is sufficiently similar to their *in vivo* development to allow both maturation and tolerance of specific CTL.

Although it has been assumed for some time that the 'tolerizing' antigen-presenting cell (APC) in the thymus is a bone marrow-derived dendritic cell<sup>15</sup>, a VETO cell<sup>5</sup> or a double-

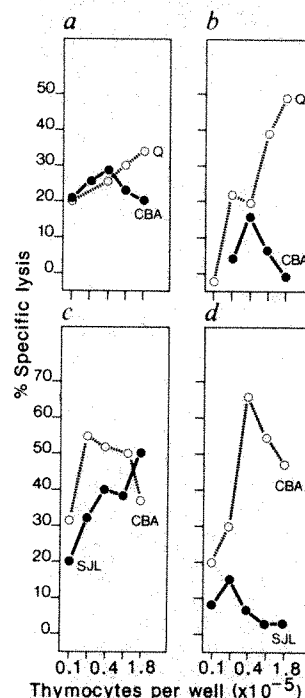


FIG. 1 Tolerance *in vitro*. Details of the mouse strains are shown in Table 1. We removed thymuses from 14-day (B6 × BALB/c) mouse fetuses and cultured them on millipore filters at the liquid-air interface in Iscove's modified Dulbecco's medium with 10% fetal calf serum, antibiotics and  $6 \times 10^{-5}$  M  $\alpha$ -thioglycerol<sup>11</sup>. The thymuses were either cultured alone (a, c; 8–10 per filter) or around and in contact with a slice (~1 mm thick) of irradiated (1,000 rads) adult CBA (b) or SJL (d) thymus. Activation cultures and cytotoxic assay: After 7 days thymocytes were collected and graded (indicated as numbers of thymocytes per well) into round-bottomed 96-well microtitre plates with  $5 \times 10^5$  irradiated (3,000 rad) adult spleen stimulator cells in medium including 10% supernatant from concanavalin-A activated rat spleen cells plus 20 mM  $\alpha$ -methylmannoside. After 6 days the medium was removed and replaced with 0.2 ml medium containing  $10^4$   $^{51}\text{Cr}$ -labelled targets (spleen cells cultured for 2 d with  $2 \mu\text{g ml}^{-1}$  concanavalin A). Released  $^{51}\text{Cr}$  was collected after 3.5 h. Cytotoxicity (per cent) was averaged from triplicate cultures and calculated from: (experimental release – spontaneous release)/(maximum release – spontaneous release)  $\times 100$ . Lytic activity is shown against the stimulating cell type. The activity on inappropriate targets was never >20% of the specific activity and was usually <1%. Occasionally (~1/8 experiments) the level of nonspecific cytotoxicity is anomalously high and so these experiments are excluded. The somewhat unusual shape of the cytotoxicity curves probably results from two factors: at low cell numbers we are at limiting dilution conditions and single active clones can sporadically appear to generate unusual peaks of activity; also, in a very active response, high cell numbers will deplete the culture medium and die before the assay. We would therefore see efficient CTL activity only at lower inputs. a, c, Thymuses cultured alone; b, co-cultured with CBA; d, co-cultured with SJL.

negative thymocyte<sup>16</sup>, it is not known whether the tolerizing cell must come from the thymus or whether the newly developing T-cell can be rendered unresponsive after interaction with any APC. To distinguish between the two alternatives, we tested the tolerizing capacity of dendritic cells from adult spleen. In the activation of mature T cells, splenic dendritic cells are among the most efficient of APCs<sup>9</sup>. Would they activate or tolerize newly developing thymocytes?

Figure 2a shows the responses of (B6 × BALB/c) thymocytes which have developed alone or in the presence of graded numbers of irradiated dendritic cells from the spleens of adult CBA mice. Thymocytes that developed alone generated good CTL against both CBA and C3H.Q, whereas thymocytes that developed in the presence of even small numbers of CBA dendritic cells responded to C3H.Q but were no longer respon-

TABLE 1 Details of mouse strains

Strain	Abbreviation	H-2 type
C57Bl/6	B6	b
BALB/c	C	d
C57Bl/6 × BALB/c	(B6 × C)	b × d
CBA	CBA	k
C3H	C3H	k
C3H.Q	Q	q
SJL	SJL	s

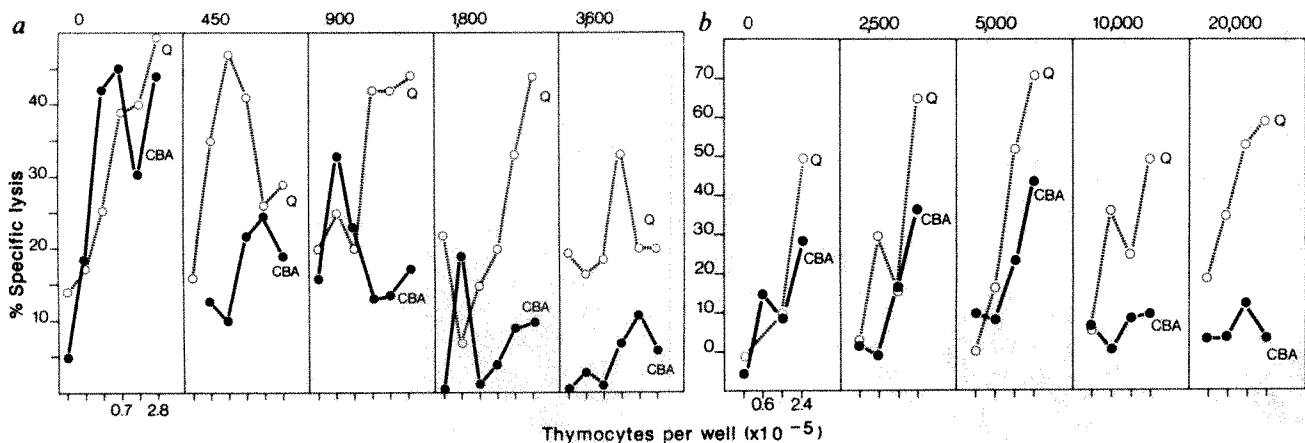


FIG. 2 Dendritic cells induce tolerance *in vitro* and *in vivo*. *a*, Thymic organ culture *in vitro*. Each 14-day fetal thymus lobe was cultured in a 20  $\mu$ l Terasaki well (upside down) in the presence or absence of graded numbers of irradiated (1,000 rads) CBA dendritic cells (20 thymus lobes per group). After 18 h each lobe was rinsed, transferred to a Millipore filter, and cultured for another 6 d. For the activation culture the 20 lobes in each group were pooled, activated and tested as described in Fig. 1 legend. The number of dendritic cells per lobe is given above each panel. *b*, Thymic organ culture *in vivo*. Thymuses were cultured overnight with CBA dendritic cells. They were then rinsed and grafted under the left kidney capsule of adult SCID mice

(8–10 lobes per mouse, 2 mice per group). After 11 days the thymocytes were collected, pooled, activated and tested. Isolation of dendritic cells: 1–2  $\times 10^8$  spleen cells per dish were cultured in 5 ml Petri dishes for 2 h, stringently washed free of all non-adherent cells. The remaining cells were then re-cultured for 18 h and the non-adherent cells gently collected. The yield varied from 0.1–0.3% of input, contained no cells positive for Thyl or immunoglobulin and <0.01% Fc receptor-positive cells. In our hands these dendritic cells were 20–30 fold more effective than spleen cells at presenting antigen to T-cell clones.

sive to CBA. Figure 2b shows that dendritic cells have the same effect *in vivo*. We added graded numbers of CBA dendritic cells, cultured the thymuses overnight and then allowed them to develop for 11 days under the kidney capsules of SCID mice (which cannot generate their own T or B cells<sup>17</sup>). Again, the presence of adult spleen dendritic cells specifically abolished responsiveness.

The number of dendritic cells which induce unresponsiveness is small and is correlated with the size of the thymus. *In vitro* the thymuses develop 2–5  $\times 10^5$  thymocytes per lobe, whereas *in vivo* the same thymuses will enlarge to 1–4  $\times 10^6$ . This 10-fold difference is reflected in the number of allogeneic dendritic cells needed for tolerance. On the average, complete tolerance is achieved with one dendritic cell per 200 thymocytes *in vitro* and *in vivo*. In tissue sections (not shown) we found that, like dendritic cells in normal thymus<sup>18</sup>, our dendritic cells do not distribute randomly throughout the thymus, but seem to collect into tubules or clusters in the medulla and at the cortico-medullary junction. Perhaps young T cells must run a gauntlet of dendritic cells before they are allowed to leave the thymus.

Figure 3 indicates that other cell types are not as effective as dendritic cells. It takes 1–2  $\times 10^3$  dendritic cells to remove 90% of the thymocyte responsiveness against them, but 100-fold more spleen cells and 1,000-fold more thymocytes are required to achieve the same effect. This difference correlates well with the frequency of dendritic cells in each population<sup>9</sup>, implying that antigen presentation for tolerance, like antigen presentation for activation, is best done by a typical APC, and that dendritic cells, the most effective activators of mature T cells, are also the most effective inactivators of young thymocytes.

The tolerizing ability of dendritic cells could solve two long standing problems. The first involves tolerance to peripheral tissue-specific antigens which are not expressed by the thymus<sup>19,20</sup>. It cannot be argued that the thymus specializes in the expression of each possible self-antigen, because tetraparental embryo-fusion chimaeras express antigens on their syncytiated muscle cells that cannot be expressed elsewhere (including in the thymus)<sup>19</sup>, and yet these mice are tolerant of their muscle. Since peripheral APCs circulate through the thymus<sup>21</sup>, they could well pick up a variety of antigens on the way, which they could then present to the developing thymocytes, thereby generating at least some level of tolerance to peripheral antigens.

Of course this does not exclude the existence of peripheral suppressive 'fail-safe' mechanisms to ensure self-tolerance should thymic depletion be imperfect<sup>32</sup>. The second problem concerns extrathymic differentiation<sup>22</sup>. Nude mice, for example, have no thymuses, yet small numbers of T cells can be found. These T cells can be activated against allogeneic and haptenated targets<sup>23</sup> but they are totally tolerant of self. If the decision to respond or be turned off is actually made by the developing T-cell rather than by the APC, then any T cells developing outside the thymus will also be rendered self-tolerant. Thus the system provides a safety valve that allows for extrathymic differentiation of T cells (and for potential evolutionary side-steps).

Tolerance induction by normal peripheral APCs also allows for conservation of specificity. We know that tolerance, like

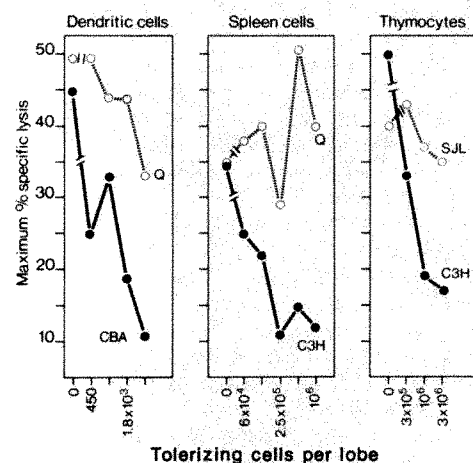


FIG. 3 Comparison of the effect of different cell populations. 14-day (B6  $\times$  BALB/c) thymuses were cultured with graded numbers of irradiated adult CBA spleen or thymus cells or purified dendritic cells as described in Fig. 1 legend. The ordinate represents the highest level of cytotoxicity seen in the titrated culture of each (pooled) group of thymocytes; the abscissa represents the number of 'tolerizing' cells per lobe. For comparison, the first panel is the same experiment as that shown in Fig. 2a. The highest point in each panel of Fig. 2a becomes one point on the curve here.



activation, is MHC-restricted<sup>24,25</sup>, and that antigen-processing is probably involved<sup>26,27</sup>. According to Bretscher and Cohn<sup>28</sup> 'every inducible cell must be tolerizable' and it should be tolerizable by the same antigen, at the same concentration and presented in the same way as the one to which it could be activated. If T cells are normally activated by peripheral APCs then it makes sense to tolerize them using the same APCs.

Here we have established that tolerance is not uniquely the result of antigen presentation by a special set of thymic antigen-presenting cells. Young thymocytes can be turned off when they recognize antigen on the same types of APCs that activate mature T cells. It could be argued that the peripheral dendritic cell might change its role on entering the thymus, turning from an activator into a tolerizer. Two sets of results stand against this. First, APCs isolated from the thymus can stimulate T-cell clones<sup>29</sup>. Second, mature T cells in the thymus can be primed by a peripheral injection of spleen cells<sup>30</sup>. We favour the view that the decision for tolerance, as opposed to activation, is made by the T cells themselves at different stages of their development. Whether the young thymocytes are innately suicidal or in need of critical, but unavailable, growth factors<sup>31</sup> remains to be investigated. □

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## Positive selection of CD4<sup>+</sup>CD8<sup>+</sup> T cells in the thymus of normal mice

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THE diversification of the repertoire of T-cell antigen receptor (TCR) specificities is influenced by at least two selection processes which occur in the thymus<sup>1–6</sup>. One of these, termed 'negative selection', is required to install a state of tolerance to self-antigens in the T-cell repertoire and is often achieved by clonal deletion<sup>7–11</sup>. The second type of selection operating in the thymus results in preferential differentiation of T cells that have restriction specificity for thymic major histocompatibility complex glycoproteins, but the mechanisms leading to this selective process are not yet clear. One model used to describe this 'positive selection' proposes that only those T cells with sufficient avidity for the MHC glycoproteins expressed in the thymus are allowed to acquire functional competence<sup>1–4,12,13</sup>. Here we directly investigate the generation of TCR specificities by following the fate of developing Vβ17<sup>+</sup> CD4<sup>+</sup>CD8<sup>+</sup> T cells under conditions where one of the main class I-MHC molecules, either H-2K or H-2D, was specifically blocked by *in vitro* monoclonal antibody treatment<sup>14</sup>. The results show that development of Vβ17<sup>+</sup> CD4<sup>+</sup>CD8<sup>+</sup> T cells in the SJL H-2<sup>s</sup> mouse strain is selectively abrogated by blocking class I-K<sup>s</sup> molecules but is unaffected by blocking class I-D<sup>s</sup> molecules. These data directly demonstrate that generation of CD4<sup>+</sup>CD8<sup>+</sup> T cells expressing a particular TCR Vβ segment can be correlated with the expression of a particular class I-MHC molecule, thereby providing evidence for positive selection.

The TCR-Vβ17a product confers reactivity to various allelic forms of the murine class II-MHC molecule I-E (refs 7 and 15)

and is differentially expressed among the CD4<sup>+</sup>CD8<sup>+</sup> and CD4<sup>+</sup>CD8<sup>+</sup> T-cell subsets of different mouse strains. For instance, SWR (H-2<sup>q</sup>) mice express Vβ17a predominantly in CD4<sup>+</sup>CD8<sup>+</sup> T cells, whereas SJL (H-2<sup>s</sup>) mice express Vβ17a in both CD4<sup>+</sup>CD8<sup>+</sup> and CD4<sup>+</sup>CD8<sup>+</sup> T cells<sup>7,16</sup>. One possible explanation for this is that the class I-MHC glycoproteins of the H-2<sup>s</sup> haplotype, but not those of the H-2<sup>q</sup> haplotype, allow for positive selection of the Vβ17<sup>+</sup> CD4<sup>+</sup>CD8<sup>+</sup> T cells. An inference from this hypothesis is that blocking one of the H-2<sup>s</sup>-class I MHC molecules could prevent the generation of Vβ17<sup>+</sup> CD4<sup>+</sup>CD8<sup>+</sup> T cells in H-2<sup>s</sup> mice without affecting the overall development of CD4<sup>+</sup>CD8<sup>+</sup> T cells.

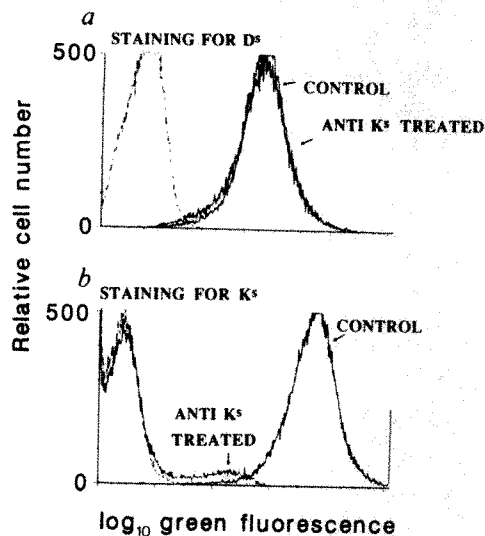
To test this prediction, we treated SJL (H-2<sup>s</sup>) mice from the day of birth with anti-class I monoclonal antibody (mAb) against either K<sup>s</sup> (mAb 34-1-2; ref. 17; mouse γ2a) or D<sup>s</sup> (mAb 15-1-5; ref. 18; mouse γ2b). The doses used (500 µg per gram body weight administered daily until analysis at 2–3 weeks of age)<sup>14</sup> were sufficient to saturate the appropriate class I-MHC on spleen

TABLE 1 Expression of Vβ17 by thymocytes from control, anti-K<sup>s</sup>-treated, and anti-D<sup>s</sup> treated H-2<sup>s</sup> mice

Treatment	Vβ17a in CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>+</sup> cells	Vβ17a in CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>+</sup> cells
Experiment 1		
Control	5.7	23.5
Anti-K <sup>s</sup>	0.3	23.9
Anti-D <sup>s</sup>	5.9	22.6
Experiment 2		
Control	5.4	20.9
Anti-K <sup>s</sup>	0.1	22.3
Anti-D <sup>s</sup>	4.8	22.4
Experiment 3		
Control	4.8	19.2
Anti-K <sup>s</sup>	0.1	23.2
Anti-D <sup>s</sup>	4.6	20.6

Mice were treated with anti-K<sup>s</sup> or anti-D<sup>s</sup> mAb as for Fig. 1. Thymocytes were enriched for receptor-bearing single positive cells by treatment with M1/69 plus complement as described in Fig. 2. Data are presented as percent positive cells (values for control-staining subtracted).

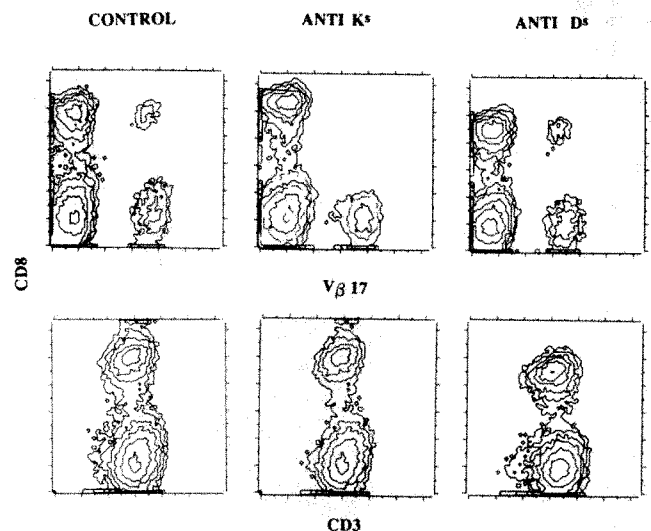
\*To whom correspondence should be addressed.



**FIG. 1** Flow cytometry analysis of cell-surface class I-H-2D<sup>s</sup> and class I-H-2K<sup>s</sup> expression on thymocytes from control and anti-K<sup>s</sup>-treated mice. Cells were stained with anti-D<sup>s</sup>-mAb (a, solid lines) or anti-K<sup>s</sup>-mAb (b, solid lines). Staining with irrelevant control mAb (dashed lines) is shown for only one of the cell samples (control or anti-K<sup>s</sup>-treated). **METHODS.** Neonatal SJL (H-2<sup>s</sup>) mice were treated within 24 h of birth with anti-K<sup>s</sup> mAb (hybridoma 34-1-2, ref. 17; reactive with K<sup>s</sup>D<sup>s</sup> and cross-reactive with K<sup>s</sup>) or anti-H-2D<sup>s</sup> mAb (hybridoma 15-1-5, ref. 18; reactive with K<sup>s</sup>D<sup>s</sup> and cross-reactive with D<sup>s</sup>). Hybridomas were obtained from ATCC (American Type Culture Collection) and purified from ascites by ammonium sulphate precipitation followed by size separation. Mice were injected daily with 500 µg mAb per gram body weight and their thymocytes analysed at 2–3 weeks of age in 4 separate experiments. Thymocytes were pretreated *in vitro* with mAb M1/69 (ref. 20) plus complement to enrich for mature T cells (see Fig. 2), and stained with either fluorescein isothiocyanate-labelled 15-1-5 (anti-D<sup>s</sup>) mAb (a) or biotinylated 34-1-2 (anti-K<sup>s</sup>) mAb, followed by allophycocyanin-labelled avidin (Caltag, San Francisco) (b). Flow cytometry analysis was performed on a B-D Dual Laser 440 interfaced to a PDP 11/24 computer<sup>14</sup>. Data were collected on 50,000 viable cells and are shown for control and anti-K<sup>s</sup>-treated mice. Reciprocal results are obtained in anti-D<sup>s</sup> treated mice, in that D<sup>s</sup>-staining is reduced and K<sup>s</sup>-staining is normal (data not shown). Staining with appropriate anti-mouse IgG antibody revealed that reduced staining was due to blocking by mAb (not shown). Control mice were treated with saline, once preliminary experiments had shown<sup>14</sup> that daily injection with equally high amounts of non-binding anti-class I mAb had no effect.

cells (data not shown) and thymocytes (Fig. 1). Flow cytometry analysis of anti-K<sup>s</sup>-treated mice revealed there had been a strong blocking of K<sup>s</sup> while D<sup>s</sup>-expression was left undisturbed. Reciprocal results, with D<sup>s</sup>-blocking and normal K<sup>s</sup> expression, were observed in anti-D<sup>s</sup>-treated mice (data not shown).

We next investigated Vβ17a expression in developing thymocytes from H-2<sup>s</sup> mice treated from birth with anti-K<sup>s</sup> or anti-D<sup>s</sup>. Although treatment of newborn homozygous mice with anti-class I mAb specific for both H-2K<sup>s</sup> and H-2D<sup>s</sup>-encoded glycoproteins prevents generation of most CD4<sup>+</sup>CD8<sup>+</sup> T cells<sup>14</sup>, treatment of newborn F<sub>1</sub> mice with anti-class I mAbs that are specific for only one of the two parental haplotypes has no effect on the total number of CD4<sup>+</sup>CD8<sup>+</sup> T cells<sup>14</sup>. By analogy, treatment of homozygous mice with anti-class I mAb specific only for either H-2K<sup>s</sup> or H-2D<sup>s</sup>-region-encoded products has no effect on overall development of mature CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> T cells<sup>19</sup> (see also Fig. 2). Thus, selective anti-K<sup>s</sup> or anti-D<sup>s</sup> mAb treatment of H-2<sup>s</sup> mice will allow for TCR-repertoire analysis of developing CD4<sup>+</sup>CD8<sup>+</sup> T cells. To enrich for TCR-bearing cells, thymocytes from control or treated mice were subjected to M1/69 (ref. 20) plus complement *in vitro* before analysis of TCR expression. The M1/69 mAb recognizes an epitope on the same molecule as the JIId mAb (ref. 21) and eliminates almost all CD4<sup>+</sup>CD8<sup>+</sup> thymocytes (see also Fig. 2) and a subset of CD4<sup>+</sup>CD8<sup>+</sup> thymocytes<sup>22</sup>. Anti-D<sup>s</sup>-mAb treatment has no effect



**FIG. 2** Two-colour flow cytometry analysis of cell-surface CD8 expression versus Vβ17a-TCR (top) or CD3 (bottom) on thymocytes from SJL (H-2<sup>s</sup>) mice which were untreated (left), treated from birth with anti-K<sup>s</sup> mAb (middle), or treated from birth with anti-D<sup>s</sup> mAb (right). Cells were stained first with FITC-conjugated anti-Vβ17 mAb or FITC-conjugated anti-CD3 mAb, then washed and stained with biotinylated anti-CD8 mAb followed by allophycocyanin-labelled avidin.

**METHODS.** Mice were treated with anti-K<sup>s</sup> or anti-D<sup>s</sup> mAbs until they were 3 weeks old, as described in Fig. 1. Thymocytes were treated with mAb M1/69 (ref. 20) plus complement to enrich for mature T cells, and stained for Vβ17a-expression with FITC-conjugated KJ23 (ref. 15) or for CD3-expression with FITC-conjugated 145-2C11 (ref. 32). Cells were then washed and stained for CD8 with biotinylated 53.6 mAb. Data were collected as described in Fig. 1 and displayed as contour diagrams. Per cent Vβ17a-expressing cells: for CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> cells, 5.7% in control; <0.5% in anti-K<sup>s</sup>-treated; 5.9% in anti-D<sup>s</sup>-treated. Per cent Vβ17a-expressing cells in CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> cells: 23.5% in control; 23.9% in anti-K<sup>s</sup>-treated; 22.6% in anti-D<sup>s</sup>-treated; these last values were derived from Vβ17a versus CD4 staining (not shown).

on Vβ17a-expression in either one of the subsets of mature T cells (Fig. 2, top). In striking contrast, thymocytes from anti-K<sup>s</sup> treated mice exhibited virtually no Vβ17a-expression in the CD4<sup>+</sup>CD8<sup>+</sup> subset (Fig. 2, top) (<0.5% in treated versus 5.7% in control); a summary of three experiments is shown in Table 1. The effect of the anti-K<sup>s</sup> treatment on Vβ17a-TCR bearing CD4<sup>+</sup>CD8<sup>+</sup> cells was specific in that it did not affect the total number of CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> T cells (Fig. 2, bottom), nor did it have any effect on the generation of Vβ17a<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> cells (Fig. 2, top; 23.9% in treated versus 23.5% in control). This highly selective perturbation of the TCR-repertoire of CD4<sup>+</sup>CD8<sup>+</sup> T cells is strong evidence that development of CD4<sup>+</sup>CD8<sup>+</sup> T cells with a certain TCR requires expression of particular alleles of the class I-MHC glycoproteins. These findings indicate that TCR-MHC ligand interactions provide signals essential to the differentiation of precursor T cells, and are evidence in favour of the positive-selection theory.

Our results on T-cell repertoire development in anti-class I-treated normal mice confirm the proposed thymic selection inferred indirectly from chimera models<sup>1–4</sup>. The data are also consistent with the observation that anti-class II-treated F<sub>1</sub> mice, in which the mAb can react with only one of the parental haplotypes, express a significantly altered repertoire<sup>23</sup>, with T-helper cells showing self-class II restriction for the unblocked class II-molecules only. In a third model system using αβ-TCR-transgenic mice<sup>10,24–26</sup>, positive selection in the context of thymic MHC also occurs, a result which together with ours shows the importance of TCR-MHC interactions in the development of T cells. Our data also bear on another aspect of TCR-repertoire selection, namely the degree to which certain TCR V gene elements can independently account for preferential recognition

of particular alleles of MHC glycoproteins. Several of the murine V $\beta$  genes so far examined have, when expressed on CD4<sup>+</sup>CD8<sup>+</sup> T cells, been associated with recognition of particular class II MHC molecules and self-antigens<sup>7-9,27-29</sup>. The observation that V $\beta$ 17a-bearing CD4<sup>+</sup>CD8<sup>+</sup> T cells are not generated in anti-K<sup>s</sup>-treated H-2<sup>s</sup> mice leads to the prediction that V $\beta$ 17a alone might confer restriction specificity for K<sup>s</sup>, regardless of the V $\alpha$  sequences expressed on its partner chain and of junctional sequences. Thus, all V $\beta$ 17a<sup>+</sup> CD4<sup>+</sup>CD8<sup>+</sup> T cells are expected to be K<sup>s</sup>-restricted. Although this prediction can be tested by analysing the restriction specificities of various antigen-specific V $\beta$ 17a-bearing CD4<sup>+</sup>CD8<sup>+</sup> T cells derived from H-2<sup>s</sup> mice, we have not yet been able to generate such cells, presumably because the vast number of potential foreign antigens precludes an easy definition of the appropriate ones. Nonetheless, the most straightforward explanation of our results is that V $\beta$ 17a<sup>+</sup> CD4<sup>+</sup>CD8<sup>+</sup> T cells are preferentially selected on K<sup>s</sup>, or by K<sup>s</sup> complexed to self-peptides<sup>30,31</sup>. Another explanation arises if we consider the opposite possibility that expression of certain TCR V gene elements precludes recognition of particular MHC molecules. According to such a model, all V $\beta$ 17a CD4<sup>+</sup>CD8<sup>+</sup> are apparently selected on K<sup>s</sup> not because V $\beta$ 17a 'dictates' K<sup>s</sup> recognition, but because a TCR with V $\beta$ 17a cannot interact with D<sup>s</sup>; complexing of D<sup>s</sup> with those self-peptides allowing for interactions with V $\beta$ 17a (refs 30 and 31) could be inefficient. We consider this hypothesis attractive because other class I-gene products seem to be 'nonpermissive' for interactions with V $\beta$ 17a: H-2<sup>q</sup> mice express V $\beta$ 17a predominantly in CD4<sup>+</sup>CD8<sup>+</sup> cells, but do not express self-genes leading to V $\beta$ 17a<sup>+</sup>-deletion in the CD4<sup>+</sup>CD8<sup>+</sup> population (that is, (SWR $\times$ SJL) F<sub>1</sub> mice (H-2<sup>q</sup> $\times$ H-2<sup>s</sup>) do not delete V $\beta$ 17a<sup>+</sup> CD4<sup>+</sup>CD8<sup>+</sup> T cells; unpublished observations). This is most easily explained by postulating that K<sup>q</sup> and D<sup>q</sup> do not allow for interactions with V $\beta$ 17a, so V $\beta$ 17a CD4<sup>+</sup>CD8<sup>+</sup> T cells cannot be positively selected. We therefore suggest that the intrinsic lack of affinity of a certain TCR V $\beta$  gene for particular MHC molecules may sometimes 'skew' the developing TCR repertoire and generate an apparent rather than a real preference for recognition of other MHC glycoproteins. □

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## A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor

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ATRIAL natriuretic peptide (ANP) is a polypeptide hormone whose effects include the induction of diuresis, natriuresis and vasorelaxation<sup>1</sup>. One of the earliest events following binding of ANP to receptors on target cells is an increase in cyclic GMP concentration, indicating that this nucleotide might act as a mediator of the physiological effects of the hormone<sup>2,3</sup>. Guanylate cyclase exists in at least two different molecular forms: a soluble haem-containing enzyme consisting of two subunits<sup>4,5</sup> and a non-haem-containing transmembrane protein having a single subunit<sup>6</sup>. It is the membrane form of guanylate cyclase that is activated following binding of ANP to target cells<sup>3,7,8</sup>. We report here the isolation, sequence and expression of a complementary DNA clone encoding the membrane form of guanylate cyclase from rat brain. Transfection of this cDNA into cultured mammalian cells results in expression of guanylate cyclase activity and ANP-binding activity. The ANP receptor/guanylate cyclase represents a new class of mammalian cell-surface receptors which contain an extracellular ligand-binding domain and an intracellular guanylate cyclase catalytic domain.

Using as probe a cDNA encoding the membrane form of guanylate cyclase from the sea urchin *Arbacia punctulata*<sup>6</sup>, we isolated partial-length putative guanylate cyclase clones from

FIG. 1 Nucleotide sequence and predicted amino acid sequence of rat brain guanylate cyclase cDNA. Nucleotides and amino acids are numbered on the left. Putative signal and transmembrane sequences are underlined, as is the polyadenylation signal. Six potential N-glycosylation sites in the putative extracellular domain are indicated by dashed lines under the amino-acid sequences. The six cysteine residues in the putative extracellular domain are boxed, as are the initiation codon, two upstream ATG sequences and four upstream stop codons.

METHODS. An oligo(dT)-primed, size-selected (>2 kilobases) library was constructed in phage  $\lambda$ gt11, using mRNA from adult rat brain. Partial-length guanylate cyclase cDNA clones were isolated from human cDNA libraries (D.G.L. *et al.*, submitted) using a sea urchin guanylate cyclase cDNA probe<sup>6</sup>. These partial-length cDNA inserts were then used as probes to screen the amplified rat brain  $\lambda$ gt11 library, grown in *Escherichia coli* Y1088 cells. Plaques were transferred to nylon filters and hybridized to random-primed, radiolabelled probes in 2 $\times$ SSC, 0.1% SDS, 5 $\times$ Denhardt's solution, 100  $\mu$ g ml<sup>-1</sup> salmon sperm DNA, at 65 $^{\circ}$ C; filters were washed with 2 $\times$ SSC, 0.1% SDS at 65 $^{\circ}$ C. The longest clone obtained was chosen for sequence analysis. The two cDNA fragments obtained by *Eco*RI digestion of the  $\lambda$ gt11 clone were subcloned into the *Eco*RI site of Bluescript KS<sup>+</sup> (Stratagene) for restriction mapping, then the appropriate restriction fragments were subcloned into M13mp18 and M13mp19 for dideoxy sequencing using Sequenase (United States Biochemical). In addition, deletions were made from the 3' end of the 5' 1.2-kilobase *Eco*RI fragment in Bluescript, as a portion of this fragment lacked convenient restriction sites for subcloning into M13. The Erase-a-Base kit (Promega) was used for the exonuclease III/SI nuclease treatments. Single-stranded templates for sequencing were rescued from the deletion clones in Bluescript using M13K07 as helper phage.



1 AAGCGCTCTCGGCTCTCGGACGCTCCCAATTGAGCGCTCTGCTCGACGGCCGAACCGTCGACGCTCCGAGGACGCGTCCGCTCGGGGTTGCGGCTTCAACCCACCCGAGCTTCTCCTCGCTACGACTCGGGCGCCCTGGACGTTCCGACCTCGC

180 CGGTGAGCCCGAGGATGCGAGCAGACCTGCTGACGCTGCCGGTGGCTGCACTCGTGGGCC ATG CCG GGC TCC CGA CGC GTC CGT CCG CGC CTA AGG GCG CTG CTG CTG CCG CCG CTT CTG CTA  
Met Pro Gly Ser Arg Arg Val Arg Pro Arg Leu Arg Ala Leu Leu Leu Pro Pro Leu Leu Leu

284 CTC CGG GGC GGC CAC GCG AGC GAC CTG ACC GTG GCT GTG GTG CTG CCG CTG ACC AAC ACC TCG TAC CCG TGG TCC TGG GCG CGT GTA GGG CCG GCC GTG GAA CTG GCT CTC GCG CCG GTG  
Leu Arg Gly Gly His Ala Ser Asp Leu Thr Val Ala Val Val Leu Pro Leu Thr Asn Thr Ser Tyr Pro Trp Ser Trp Ala Arg Val Gly Pro Ala Val Glu Leu Ala Leu Ala Arg Val

414 AAG GCT CGG CCG GAC TTG CTG CCG GGT TGG ACG GTC CGC ATG GTG CTG GGC AGC AGT GAG AAC GCG GCG GGC GTC TGC TCG GAC ACC GCC GCA CCG CTG GCC GCG GTG GAC CTC AAG TGG  
Lys Ala Arg Pro Asp Leu Leu Pro Gly Trp Thr Val Arg Met Val Leu Gly Ser Ser Glu Asn Ala Ala Gly Val Cys Ser Asp Thr Ala Ala Pro Leu Ala Ala Val Asp Leu Lys Trp

534 GAG CAC AGC CCC GCG GTG TTC CTG GGC CCC GGC TGC GTC TAC TCC GCT GCC CCG GTG GGG CCG TTC ACC GCG CAC TGG CCG GTG CCG CTG CTG ACC GCC GGC GCC CCG GCT CTG GGC ATC  
Glu His Ser Pro Ala Val Phe Leu Gly Pro Gly Cys Val Tyr Ser Ala Ala Pro Val Gly Arg Phe Thr Ala His Trp Arg Val Pro Leu Leu Thr Ala Gly Ala Pro Ala Leu Gly Ile

654 GGG GTC AAG GAT GAG TAT GCG CTA ACC ACC CGC ACA GGA CCC AGC CAT GTC AAG CTG GGC GAT TTC GTG ACG GCG CTG CAT CGA CCG CTG GGC TGG GAG CAC CAG GCG CTG GTG CTC TAT  
Gly Val Lys Asp Glu Tyr Ala Leu Thr Thr Arg Thr Gly Pro Ser His Val Lys Leu Gly Asp Phe Val Thr Ala Leu His Arg Arg Leu Asp Gly Trp Glu His Glu Ala Val Asp Leu Thr Tyr

774 GCA GAT CCG CTG GGC GAC GAC CCG CDT TGC TTC ATA GTG GAG GGG CTG TAC ATG CCG GTG CGT GAA CGC CTC AAC ATC ACA GTG AAT CAC CAG GAG TTC GTG GAG GGC GAC CCG GAC  
Ala Asp Arg Leu Gly Asp Asp Arg Pro Cys Phe Phe Ile Val Glu Gly Leu Tyr Met Arg Val Arg Glu Arg Leu Asn Ile Thr Val Asn His Glu Glu Phe Val Glu Gly Asp Pro Asp

894 CAC TAC CCC AAG CTA CTG CCG GCC GTG CCG CGA AAG GGC AGA GTT ATC TAC ATC TGC AGT TCT CCG GAT GCC TTC AGG AAT CTG ATG CTT CTG GCC CTG AAC GCT GGC CTG ACT GGG GAG  
His Tyr Pro Lys Leu Leu Arg Ala Val Arg Arg Lys Gly Arg Val Ile Tyr Ile Cys Ser Ser Pro Asp Ala Phe Arg Asn Leu Met Leu Leu Ala Leu Asn Ala Gly Leu Thr Gly Glu

1014 GAC TAT GTT TTC TTC CAC CTG GAT GTG TTT GGG CAA AGC CTT AAG AGT GCT CAG GGC CTT GTT CCC CAG AAA CCC TGG GAA AGA GGA GAT GGG CAG GAC AGG AGT GCC CGC CAG GCC TTT  
Asp Tyr Val Phe Phe His Leu Asp Val Phe Gly Glu Ser Leu Lys Ser Ala Glu Gly Leu Val Pro Glu Lys Pro Trp Glu Arg Gly Asp Gly Glu Asp Arg Ser Ala Arg Glu Ala Phe

1134 CAG GCT GCC AAA ATT ATT ACT TAC AAA GAG CDT GAT AAT CCT GAG TAC TTG GAA TTC CTG AAG CAG CTG AAA CTC TTG GCT GAC AAG AAG TTC AAC TTC ACC GTG GAG GAT GGC CTG AAG  
Lys Glu His Ser Pro Ala Val Phe Leu Gly Pro Asp Asn Pro Gly Tyr Leu Glu Phe Leu Lys Glu Leu Lys Leu Leu Ala Asp Lys Lys Phe Asn Phe Thr Val Glu Asp Gly Leu Lys

1254 AAT ATC ATC CCA GCC TCC TTC CAC GAC GGG CTC CTG CTT TAT GTC CAG GCA GTG ACA GAG ACT CTG GCA CAG GGG GGA ACT GTC ACA GAT GGA GAG AAC ATC ACT CAG CCG ATG TGG AAC  
Asn Ile Ile Pro Ala Ser Phe His Asp Gly Leu Leu Thr Tyr Val Glu Ala Val Thr Glu Thr Leu Ala Glu Gly Gly Thr Val Thr Asp Gly Thr Val Thr Asn Ile Thr Glu Arg Met Trp Asn

1374 CGA AGC TTC CAA GGT GTG ACA GGA TAC CTG AAA ATT GAT AGA AAC GGA GAT CCG GAC ACC GAT TTC TCT CTC TGG GAT ATG GAT CCA GAG ACG GGT GCC TTC AGG GTT GTC CTG AAC TAT  
Arg Ser Phe Glu Gly Val Thr Gly Tyr Leu Lys Ile Asp Arg Asn Gly Asp Arg Asp Thr Asp Phe Ser Leu Trp Asp Met Asp Pro Glu Thr Gly Ala Phe Arg Val Val Leu Asn Tyr

1494 AAT GGT ACT TCC CAG GAG CTA ATG GCT GTG TCA GAA CAC AAA TTA TAC TGG CCT CTG GGA TAT CCA CCT CCT GAC GTC CCT AAA TGT GGC TTT GAC AAT GAG GAC CCA GCC TGC AAC CAA  
Asn Gly Thr Ser Glu Glu Leu Met Ala Val Ser Glu His Lys Leu Tyr Trp Pro Leu Gly Tyr Pro Pro Pro Asp Val Pro Lys Cys Gly Phe Asp Asn Glu Asp Pro Ala Cys Asn Glu

1614 GAC CAC TTT TCC ACA CTG GAG GTT CTG GCT TTG GTG GGC AGC CTC TCT CTG ATT AGC TTT CTG ATT GTG TCT TTC TTA TAC AGG AAG ATG CAG CTG GAA AAG GAG CTG GTC TCA GAG  
Asp His Phe Ser Thr Leu Glu Val Leu Ala Leu Val Gly Ser Leu Ser Leu Ile Ser Phe Leu Ile Val Ser Phe Phe Ile Tyr Arg Lys Met Glu Leu Glu Lys Glu Leu Val Ser Glu

1734 TTG TGG CCG GTG CCG TGG GAG GAC TTG CAG CCC AGC AGC CTG GAG AGG CAT CTT CCG AGC GGT GGC AGC CCG CTG ACC CTG AGT GGG CGA GGC TCC AAT TAT GGC TCC CTG CTA ACC ACC  
Leu Trp Arg Val Arg Trp Glu Asp Leu Glu Pro Ser Ser Leu Glu Arg His Leu Arg Ser Ala Gly Ser Arg Leu Thr Leu Ser Gly Arg Gly Ser Asn Tyr Gly Ser Leu Leu Thr Thr

1854 GAG GGC CAG TTC CAA GTC TTT GCC AAG ACA GCA TAC TAT AAG GGC AAC CTT GTG GCT GTG AAA CGT GTG AAC CCG AAA CCG ATT GAG TTG ACA CGA AAA GTC CTG TTT GAA CTT AAA CAT  
Lys Glu Gly Glu Phe Glu Val Phe His Val Thr Ala Tyr Tyr Lys Gly Asn Leu Val Ala Val Lys Arg Val Asn Arg Lys Arg Ile Glu Leu Thr Arg Lys Val Leu Phe Glu Leu Lys His

1974 ATG CCG GAT GTG CAG AAT GAG CAC TTG ACA AGA TTT GTG GGT GCT TGT ACC GAC CCC CCC AAC ATC TGT ATC CTC ACA GAG TAC TGT CCC CGT GGA AGC CTA CAG GAC ATT CTA GAG AAT  
Met Arg Asp Val Glu Asn Glu His Leu Thr Arg Phe Val Gly Ala Cys Thr Asp Pro Pro Asn Ile Cys Ile Leu Thr Glu Tyr Cys Pro Arg Gly Ser Leu Glu Asn Ile Leu Glu Asn

2094 GAG AGT ATC ACC CTG GAC TGG ATG TTT CCG TAC TCG CTC ACC AAT GAC ATT GTC AAG GGA ATG CTC TTT CTA CAC AAT GGG GCC ATT TGT TCC CAT GGG AAC CTC AAG TCA TCC AAC TGT  
Glu Ser Ile Thr Leu Asp Trp Met Phe Arg Tyr Ser Leu Thr Asn Asp Ile Val Lys Gly Met Leu Phe Leu His Asn Gly Ala Ile Cys Ser His Gly Asn Leu Lys Ser Ser Asn Cys

2214 GTG GTA GAC GGG CCG TTC GTG TTA AAG ATC ACA GAC TAC GGT CTT GAG AGC TTC AGA GAC CCG GAG CCA GAG CAA GGG CAC ACC CTC TTT GCC AAA AAA TTG TGG ACG GCA CCT GAG CTC  
Val Val Asp Gly Arg Phe Val Leu Lys Ile Thr Asp Tyr Gly Leu Glu Ser Phe Arg Asp Pro Glu Pro Glu Glu His Thr Leu Phe Ala Lys Lys Leu Trp Thr Ala Pro Glu Leu

2334 CTG CGA ATG GCT TCG CCA CCT GCC CGT GGC TCC CAA GCT GGG GAT GTG TAC AGC TTT GGT ATC ATC CTG CAG GAG ATT GCC CTA AGA AGT GGG GTC TTC TAT GTG GAA GGT TTG GAC CTC  
Leu Arg Met Ala Ser Pro Pro Ala Arg Gly Ser Glu Ala Gly Asp Val Tyr Ser Phe Gly Ile Ile Leu Glu Glu Ile Ala Leu Arg Ser Gly Val Phe Tyr Val Glu Gly Leu Asp Leu

2454 AGC CCA AAA GAG ATC ATT GAG CGT GTG ACT CCG GGT GAG CAG CCC CCA TTC CGA CCC TCC ATG GAT CTG CAG AGC CAC CTG GAG GAA CTG GGG CAG CTG ATG CAG CCG TGC TGG GCA GAG  
Lys Ser Pro Lys Glu Ile Ile Glu Arg Val Thr Arg Gly Glu Glu Pro Pro Phe Arg Pro Ser Met Asp Leu Glu Ser His Leu Glu Glu Leu Gly Glu Met Glu Arg Cys Trp Ala Glu

2574 GAC CCA CAG GAG CCG CCA CCC TTT CAG CAG ATC CCG CTG GCG CTG CCG AAG TTC AAC AAG GAG AAC AGC AGC AAC ATC CTG GAC AAC CTG CTG TCA CCG ATG GAG CAG TAT GCT AAC AAC  
Asp Pro Glu Glu Glu Pro Phe Glu Glu Thr Arg Leu Ala Leu Arg Lys Phe Asn Lys Glu Asn Ser Ser Asn Ile Leu Asp Asn Leu Ser Arg Met Glu Glu Tyr Ala Asn Asn

2694 CTG GAG GAA CTG GTA GAG GAG AGA ACA CAA GCT TAT CTG GAG GAG AAG GCG AAA GCT GAG GCC TTG CTT TAC CAG ATT CTG CCT CAC TCC GCT GAG CAG CTG AAG AGA GGC GAG ACA  
Leu Glu Glu Leu Val Glu Glu Arg Thr Glu Ala Tyr Leu Glu Glu Lys Arg Lys Ala Glu Ala Leu Leu Tyr Glu Ile Leu Pro His Ser Val Ala Glu Glu Lys Arg Gly Glu Thr

2814 GTC CAG GCT GAG GCC TTT GAT AGT GTT ACC ATC TAC TTC AGT GAT ATT GTG GGC TTT ACA GCT CTT TCA GCA GAA AGC ACA CCC ATG CAG GTG GTG ACT CTG CTC AAT GAT CTG TAC ACC  
Val Glu Glu Ala Glu Ala Phe Asp Ser Val Thr Ile Tyr Phe Ser Asp Ile Val Gly Phe Thr Ala Leu Ser Ala Glu Ser Thr Pro Met Glu Val Val Thr Leu Leu Asn Asp Leu Tyr Thr

2934 TGT TTT GAT GCT GTC ATA GAC AAC TTT GAT GTG TAC AAG GTG GAG ACC ATT GGT GAT GCT TAC ATG GTG GTG TCA GGG CTC CCA GTG CCG AAT GGA CAA CTC CAC GCC CGA GAG GTG GCC  
Cys Phe Asp Ala Val Ile Asp Asn Phe Asp Val Tyr Lys Val Glu Thr Ile Gly Asp Ala Tyr Met Val Val Ser Gly Leu Pro Val Arg Asn Gly Glu Leu His Ala Arg Glu Val Ala

3054 CGA ATG GCA CTT GCA CTA CTG GAT GCT GTG CCG TCC TTC CCG ATC CCG CAT AGG CCC CAG GAA CAG CTG CCG TTG CCG ATT GGC ATC CAC ACA GGT CCT GTG TGT GCT GGT GTG GTA GGG  
Arg Met Ala Leu Ala Leu Leu Asp Ala Val Arg Ser Phe Arg Ile Arg His Arg Pro Glu Glu Glu Leu Arg Leu Arg Ile Gly Ile His Thr Gly Pro Val Cys Ala Gly Val Val Gly

3174 CTA AAG ATG CCC CGA TAC TGC CTC TTT GGA GAC ACA GTC AAC ACA GCT TCA AGA ATG GAG TCT AAT GGA GAA GCC CTC AAG ATC CAC TTG TCT TCA GAG ACC AAG GCT GTG CTG GAA GAG  
Leu Lys Met Pro Arg Tyr Cys Leu Phe Gly Asp Thr Val Asn Thr Ala Ser Arg Met Glu Ser Asn Gly Glu Ala Leu Lys Ile His Leu Ser Ser Glu Thr Lys Ala Val Leu Glu Glu

3294 TTC GAT GGT TTC GAG CTG GAG CTC CGA GGG GAT GTG GAA ATG AAG GGC AAA GGC AAG GTT CCG ACC TAT TGG CTC CTG GGG GAG CCG GGA TGT AGC ACT CGA GGC TGA CCTACTGCCCTG  
Phe Asp Gly Phe Glu Leu Glu Leu Arg Gly Asp Val Glu Met Lys Gly Lys Gly Lys Val Arg Thr Tyr Trp Leu Leu Gly Glu Arg Gly Cys Ser Thr Arg Gly \*

3414 GTGTTCTTGTGACCCCTCTCCTGTGCGCAGAGGTGACAGAGGTGTCAGCTTCCACCTCTCCACAGCAGCCCGCACTGTGGAAGATTAGGACCTGACCCAGCAGTACCAGATGTGACCTCTGAGAGAGGATGGAGATGGTGGGACTGCA

3573 GGGGACACCTAAGTTTGTAGGACTGACTGAAACACAGTCCCTCCCATGGCACCCTGTGGCACACATGCCAGTCCCACCTTACTCTGCTGCCTAGATTGGGACAGCATTCTCTCTGCCCTCAACTAGCTCCACTGTGACTTATAGGGAGGAA

3732 TTGCCACCTGAAGGAACAGAAAGAGGTTAGAGTTTCAGGAGGCGAGGAGTCTGTGTACAAATACTCCCTCACTTCCAGCCCAACCACTGCCCCACAGACTTGGACACAGCTTGGACACAGCTCACTGAGGGAAGAGAGGCTGCCGTTACCTTGTCTCTCTG

3891 TGAACCAACCAATTAAGTCTTTATCTCTGTGAAAAA

*a*

Rat GC SDLTAVVPLTNTSYPSWARVGPVELALARVKARP...DLLPGWTVRMVLSSENAAGVCSDTAAPLAADVLEKWEHSPAVFLGPGCVSYAAPVGRFTA 98  
 \* \* \* \* \*

Bov. ANP-C QKIEVLVLLP-QDDSYLFLSLARVRPAIEYALRTVEGNATGRLLPA-GTRFQVAYEDSDCGN-RALFSLVDRVAAARGAKPDLILGPVCEYAAAPVARLAS 103  
 \* \* \* \* \*

Rat GC HWRVPLLTAGAPALGIGVKD-EYALTRTGPSHVKLGDFTALHRLGWEHQALVLYADRLGDDRPCFFIVEGLYMRVRERLNIYVNHQEFVEGDPDHYPK 198  
 \* \* \* \* \*

Bov. ANP-C HWDLPMLSLAGALAGFQHKDTEYSHLTRVAPSYAKGEMMLALFRHHQWSRAVLVYSDDKL-ERNCFITLEGVHEVFQEE-GLHTSAYNFDET KDLDLED 201  
 \* \* \* \* \*

Rat GC LLRAVRKRGVYIICSSPDAFRNMLLALNAGLTGEDYVFFHLDVFGQSLKSAQGLVPQKWPWEGDQDRSARQAFQAAKIIITYKEPDNPEYLEFLKQLKL 299  
 \* \* \* \* \*

Bov. ANP-C IVRHQASERVVIMCASSDITRGIMLAHRHGMTSGDYAFFNIEFNSSF-YGDG-...SWKRGDKHDFEAKQAYSSLQITLLRTVKLEFEKFSMEVKS 296  
 \* \* \* \* \*

Rat GC LADKKFNFTVEDGLKNIIPASFHDGLLLVYQAVTETLAQGGVTVDGENITQRMWNRSGQVGTGKIDRNGDRDITDFSLWDM-DPETGAFRVVLNYNGTSQ 399  
 \* \* \* \* \*

Bov. ANP-C SVEKQ-GLS-EEDYVNMFEVGFHDAILLYLALREVLRAGYSKKDGGKI IQOTWNRTEFEGAGQVSI DANGDRYGDVSIAMTDEAGTQEVIGDYFGKEG 395  
 \* \* \* \* \*

Rat GC ELMAVSEHKLYW-PLGYPPDPVKCGFDNEDPACNQDHFSTLEVLALVGSLSLSIFLVSFFIYRKMLEKELVSELWRVRWEDLPSSLERHLSAGSRL 499  
 \* \* \* \* \*

Bov. ANP-C RFEMRPNVKYPWGLKLRIDETRMVEHTNSSPKASGGLES AVTGI VVGALLGAGLLMAFYFRKKYRITIERRNOQEEENVGKHRELREDSIRSHFVA 496  
 \* \* \* \* \*

*b*

Rat GC TLSGSGSNYSLLTTEGQFQVFAKTAYYKGNLVAVKVRNKRRIELTRKVLFEKHMEDV-ONEHTRFVGACTDPPNICILTEYCPRGSLQDILENESIT 598  
 \* \* \* \* \*

Mouse PDGFR RTLGSQA-FCQVEATAHGLSHSQ-...ATMKVAVKMLKSTARSEKQALMSSELKIMSHLGPLHMYNLLGACTKGPIYIITEYCRGDLVDYLHKNHT 667  
 \* \* \* \* \*

Rat GC .....LDMMFRYSLTNDIVKMLFLHNGAICSHGNLKSSNCVVDGRFVLKIDYGL-ESFRDPE-PEQHTLFAKLTAPELLRMASPPARGSQAGD 689  
 \* \* \* \* \*

Mouse PDGFR ([98]-LSYTDLVGFSYQVANSDFL-ASKNCVHDLAARNVLCCEGLV-KICDFGLARDIMRDSNYISKGSTYLPK-MAPE-...SIFNSLYTTLS 853  
 \* \* \* \* \*

Rat GC VYSFGLQEIARSGVFYVEGLDSPKEIERVTRGEOPFRPSMDLQSHLEELGQLMQRCAEDPQEPFQOIRLALRKFKNKSSNILDNLSRMEQ 790  
 \* \* \* \* \*

Mouse PDGFR VWSFGLLWEIFTLGGTYPE-LPMN-DQFYNAIKRG-...YMAQPAHAS-DEIYEIMQKWEKFEFPPFSQVLLERLLGEGYKKYQVDEEFLR 947  
 \* \* \* \* \*

*c*

Yeast AC ENITILCLALYENIQQNRFTLNKNSLMTRRSTFEDTIRLOPAISPTGNLAVFTDIKSSTFL-...W-ELFPNARMTAKTHNDIMRQLR-...IY 1706  
 \* \* \* \* \*

Rat GC YANNLEELVEERTOAYLEEKRAEALLYQILPHSVAEQLKRGETVQAEAFDSVTIYFSDIVGFTAL-...SAESTPMQVYTLNDLYTCFDAVID-...NF 883  
 \* \* \* \* \*

Bov. Sol. GC ELEILTDLRLQLTRALEDEKKKTDTLTVSVLPSPVANELRHKRPVPAKRYDNVTILFSGIVGFNAFCSKHASGEGAMKIVNLLNDLYTRFDTLDSRKNPF 468  
 \* \* \* \* \*

Yeast AC GGYEYKTEGDAFMVAFPTPTSGLTWCLSVQLKLLDAQWPEEITSVDGQCVTDNRGNIYQGLSVRMGIHWG-CPVPELDLVTQRMVLYGPMVNAKARV 1804  
 \* \* \* \* \*

Rat GC DVYKVEITGDAYMV-...VSGLP-VRNGQLHAREVARMALALLDAVRSFRIHRPQE-...QLRLRIGIHTGPVAGV-VGLKMPRYCLFGDTVNTASRM 973  
 \* \* \* \* \*

Bov. Sol. GC -VYKVEITGDYKMT-...VSGLP-EPCH-HARSICHLALDME-IG-QV-QVDGE-...SVQITIGIHTGEVTVG-IGQRMPRYCLFGNTVNLTSRT 553  
 \* \* \* \* \*

Yeast AC QGVADGGQIAMSSDF-YSEF-NKIM-KYHERVVGKESLKEYGEEIIGEVLEREIAMLE 1861  
 \* \* \* \* \*

Rat GC ESNGEALKIHLSSET-KAVL-EEFD-QF-ELELRGDVEMKGGKVRTYVLLGERGCSTRG 1029  
 \* \* \* \* \*

Bov. Sol. GC ETTGEGKINVSEYTYRCLMTPENSDDQF-HLEHRGPVSMGKKEPMQVWFLSRKNTGTEE 613  
 \* \* \* \* \*

*d*

Rat GC IYRKMLEKELVSELWRVRWEDLPSSLERHLSAGSRLTSGSGSNYSLLTTEGQFQVFAKTAYYKGNLVAVKVRNKRRIELTRKVLFEKHMEDVQNE 561  
 \* \* \* \* \*

Ap GC IYRKRAYEALDSLWKVDWKEVQ-...TRESETNSQGSMSKSMVLSAISVISNAEKQIFATIGTYRGTICAIHAVHKNHIDLTRAVRTELKLMRDMRHD 602  
 \* \* \* \* \*

Rat GC HLTRFVGACTDPPNICILTEYCPRGSLQDILENESITLDMMFRYSLTNDIVKMLFLHNGAICSHGNLKSSNCVVDGRFVLKIDYGLSFR-...DPEPEQ 659  
 \* \* \* \* \*

Ap GC NICPFIGACIDRPHICILMHYCAKGLQDWMENDDKLDSMFLASLIADLYKGLVYLHSSEIKSHGLKSSNCVVDNRWLQITDYGLHEFRKQGEDVDL 703  
 \* \* \* \* \*

Rat GC G-HTLFAKLTAPELLRMASPPARGSQAGDVYSFGLQEIARSGVFYVEGLDSPKEIERVTRGEOPFRPSMDLQSHLEELGQLMQRCAEDP 756  
 \* \* \* \* \*

Ap GC GEHAKLARKLTAPHLREGKSMHPGGTPKGDYFSIILTEMYSRQEPFHENDLEL-ADIARVSKGEVPPYRVPVNAVNEAAPDCVLTAIRACWEDP 802  
 \* \* \* \* \*

Rat GC QERPPFQOIRLALRKFKNKSSNILDNLSRMEQYANNLEELVEERTOAYLEEKRAEALLYQILPHSVAEQLKRGETVQAEAFDSVTIYFSDIVGFTALS 857  
 \* \* \* \* \*

Ap GC MERPNIEVTRMLAPLQKGLKPNILDNMIAIMERYTNLEELVDERTQELQEKAKTEQLHRMLPSSIASQLIKGISVLPETFDVMSIFFSDIVGFLIHF 903  
 \* \* \* \* \*

FIG. 2 Alignment of deduced amino-acid sequences of the membrane form of guanylate cyclase from rat brain with a, the bovine ANP-C receptor<sup>16</sup>; b, the murine PDGF receptor<sup>17</sup>; c, bovine soluble guanylate cyclase<sup>19</sup> and yeast adenylate cyclase<sup>20</sup>; d, the membrane form of guanylate cyclase from the sea urchin *Arbacia punctulata*<sup>6</sup>. The entire sequence of the mature rat guanylate cyclase is shown in a-c. Boxed residues represent the putative transmembrane domains (a), the 33 residues most highly conserved among protein kinases<sup>18</sup> (b), and sequences extending beyond the kinase-like domain (d). Regions of identity are indicated by asterisks.

human cDNA libraries (D.G.L. *et al.*, manuscript in preparation). These clones were used in turn, to probe a size-selected rat brain cDNA library to isolate a cloned cDNA containing the entire coding region of guanylate cyclase.

The nucleotide and deduced amino-acid sequences of the guanylate cyclase cDNA are shown in Fig. 1. An open reading frame encoding 1,057 amino acids is preceded by an in-frame stop codon and is flanked by 227 base pairs (bp) and 535 bp of 5' and 3' untranslated sequences respectively. Hydrophobic analysis predicts an amino-terminal signal sequence and a single transmembrane domain that divides the protein into a 441-amino-acid extracellular domain and a 567-amino-acid intracellular domain. Using an algorithm for prediction of the signal cleavage site<sup>9</sup>, residue 29 is expected to represent the amino terminus of the mature protein, which would have a relative molecular mass ( $M_r$ ) of 115,852 and an isoelectric point of 6.33. Taking into account post-translational processing known to occur in guanylate cyclase<sup>10,11</sup>, the predicted  $M_r$  is consistent with estimates of 120,000–180,000 (120K–180K) for membrane forms of guanylate cyclase reported in the literature<sup>10–13</sup>. Six cysteine residues and six potential N-glycosylation sites are present in the extracellular domain.

Further analysis of the deduced amino-acid sequence of the guanylate cyclase cDNA indicates that it may be divided into three potential functional domains (Fig. 2a–c). The extracellular domain of the rat cell membrane guanylate cyclase is 33% identical with the bovine ANP-clearance (ANP-C) receptor, an ANP-binding protein that is apparently not coupled to activation of guanylate cyclase or to the biological effects of ANP (refs 14–16) (Fig. 2a). The ANP-C receptor consists of an extracellular ANP-binding domain, a transmembrane domain and a short cytoplasmic tail<sup>16</sup>; it has been suggested that the function of this protein is to clear excess ANP from the circulation<sup>14</sup>. The five cysteine residues of the ANP-C receptor are conserved guanylate cyclase.

As noted earlier for guanylate cyclase from sea urchin<sup>6</sup>, the intracellular domain is related to the catalytic domain of protein kinases, although protein kinase activity has not yet been detected. A 256-amino acid portion of the intracellular domain is 31% identical to the protein tyrosine kinase domain of the platelet-derived growth factor (PDGF) receptor<sup>17</sup> (Fig. 2b). Guanylate cyclase conforms to the protein kinase consensus sequence<sup>18</sup> in 30 out of 33 residues. Exceptions are Ser<sup>502</sup> (Leu, Ile or Val in all known protein kinases), Asn<sup>628</sup> (Asp in all known protein kinases), and Ala<sup>665</sup> (Ser, Thr or Pro at this position in 63 out of 65 protein kinases). In addition, the Gly-X-Gly-X-X-Gly consensus sequence of protein kinases is Gly-X-Gly-X-X-Gly in the rat guanylate cyclase.

The most extensive similarity between the deduced amino-acid sequence of the membrane form of guanylate cyclase and other proteins is found in the carboxyl portion of the intracellular domain. A 253-amino acid sequence in this region is 42% identical to the carboxyl terminus of one of the subunits of a bovine soluble form of guanylate cyclase<sup>19</sup> (Fig. 2c). It is not yet known whether the sequence determined for the soluble enzyme is from a regulatory or catalytic subunit<sup>19</sup>. But only a 77-residue portion of this 253-amino acid region is conserved in guanylate cyclase from the sea urchin *Arbacia punctulata* (see below), which implies that it may not be the guanylate cyclase

catalytic domain. This region of the carboxyl tail has limited (23%) sequence identity with a 193-amino-acid portion of the yeast adenylate cyclase<sup>20</sup> (Fig. 2c); the significance, if any, of this limited similarity is not known.

The deduced amino-acid sequence of rat brain guanylate cyclase is 45% identical to that of the membrane form of guanylate cyclase from the sea urchin *Arbacia punctulata*<sup>6</sup> within a 399-amino acid region of the intracellular domain beginning immediately after the transmembrane domain (Fig. 2d). This region consists primarily of the protein kinase-like domain but also includes 77 amino acids carboxyl to this domain (Fig. 2d). Presumably this highly conserved region includes the guanylate cyclase catalytic domain, whereas the divergent regions may represent regulatory domains.

To confirm that the isolated cDNA encoded a protein with guanylate cyclase activity, we inserted the cDNA in a mammalian expression vector (pSVL) under the control of the SV40 late promoter. Because upstream AUG codons have been reported to reduce the efficiency of translation of messenger

TABLE 1 Particulate guanylate cyclase activity and <sup>125</sup>I-labelled ANP-binding activity of transfected COS-7 cells

Transfection	Guanylate cyclase activity (pmol cGMP min <sup>-1</sup> per mg protein)		Specific <sup>125</sup> I-ANP binding (c.p.m.)
	–ANP	+ANP	
pSVL	0.38	1.1	3,082
pSVL-GC	16.3	55.8	28,039

COS-7 cells, maintained in Dulbecco's modified Eagle's medium (DME) supplemented with 20 mM HEPES at pH 7.4 with penicillin/streptomycin and 10% fetal bovine serum, were transfected with plasmid DNA using DEAE-dextran<sup>28</sup>. Cells were seeded at  $5 \times 10^6$  cells per 100-mm plate (guanylate cyclase assay) or  $5 \times 10^5$  cells per 35-mm plate (<sup>125</sup>I-labelled ANP binding assay). Cells were fed the day after transfection and assayed for guanylate cyclase activity or <sup>125</sup>I-ANP binding two days after transfection. The guanylate cyclase construct in pSVL (Pharmacia) was prepared as follows. The 5' 1.2-kb *EcoRI* fragment of the guanylate cyclase cDNA was cloned into the *EcoRI* site of Bluescript KS<sup>+</sup>, oriented so that the 5' end of the insert was closest to the *HindIII* site in the polylinker. The 194 bp at the 5' end of the insert were removed by digestion with *HindIII* and *NcoI*, and the 4-kb fragment containing the remainder of the cDNA and the vector was purified by agarose gel electrophoresis and adsorption to glass beads. The DNA was then treated with *S1* nuclease and religated. The extent of the resulting deletion was determined by double-stranded sequencing. The 3' 2.7-kb *EcoRI* fragment of the guanylate cyclase cDNA was then cloned into the *EcoRI* site of the modified plasmid, and its orientation confirmed by restriction analysis. The *SalI/SmaI* fragment containing the guanylate cyclase cDNA with the upstream ATGs removed was then cloned into the *XhoI/SmaI* site of pSVL to create pSVL-GC. For guanylate cyclase assays, 100-mm cultures on ice were washed twice with 5 ml cold HEPES-buffered saline, scraped into 3 ml cold 20 mM HEPES, pH 7.4, 50 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol, and homogenized by passage ten times through a 22-gauge needle. Following centrifugation for 15 min at 5,000g and washing with the same buffer, membrane proteins were solubilized by incubation on ice for 30 min in a solution containing 20 mM HEPES, pH 7.4, 100 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM dithiothreitol. After centrifugation for 15 min at 5,000g, supernatant fluids were adjusted to equal protein concentrations and incubated for 10 min in the absence or presence of 1  $\mu$ M ANP at 0 °C. Incubation was continued for 30 min at 30 °C after diluting 100  $\mu$ l each sample into a solution containing a final concentration of 20 mM HEPES, pH 7.4, and 1 mM each GTP, MnCl<sub>2</sub> and methyl isobutylxanthine, in a total reaction volume of 250  $\mu$ l. Reactions were stopped by the addition of 750  $\mu$ l 0.5 M perchloric acid and samples were analysed for cGMP by radioimmunoassay after purification of nucleotides by Dowex chromatography<sup>26</sup>. <sup>125</sup>I-labelled ANP binding was measured essentially as described<sup>15</sup>. Cells in 35-mm plates were washed twice with 2 ml DME/0.1% bovine serum albumin (BSA), then incubated in 0.5 ml the same buffer containing 0.5 nM <sup>125</sup>I-labelled rat ANP (2,200 Ci mmol<sup>-1</sup>, NEN) in the absence or presence of 0.5  $\mu$ M unlabelled rat ANP (Sigma) for 20 min at 37 °C. After five 1-ml washes with HBSS/0.1% BSA, cells were solubilized in 1 ml 0.5 M NaOH and the radioactivity determined. All determinations were in triplicate (nonspecific binding was ~5,000 c.p.m. in each case).



RNA<sup>21,22</sup>, 194 bp of 5' untranslated sequences were removed from the cDNA before its insertion into pSVL; this resulted in the deletion of the two ATG sequences 5' to the initiation site (see Fig. 1 and Table 1 legend). This modification of the cDNA was found to enhance expression of guanylate cyclase about tenfold in transfected mammalian cells (data not shown). COS-7 cells were transfected with the guanylate cyclase expression plasmid (pSVL-GC) or with pSVL, and a particulate fraction was isolated from the cells and assayed for enzyme activity. The activity of membrane-associated guanylate cyclase was increased by about 40-fold in cells transfected with pSVL-GC (Table 1), which is consistent with the clone encoding guanylate cyclase. ANP stimulated guanylate cyclase activity about threefold in detergent extracts of both control cells and cells expressing the cloned guanylate cyclase (Table 1).

The cloned guanylate cyclase also functions as an ANP-receptor (Table 1). Cells transfected with pSVL-GC specifically bound about nine times more <sup>125</sup>I-labelled ANP than cells transfected with pSVL alone. <sup>125</sup>I-labelled ANP bound with high affinity and the expected specificity (half-maximal inhibition of binding of 0.4 nM <sup>125</sup>I-labelled ANP occurred at about 3 nM unlabelled ANP or 100 nM unlabelled atriopeptin I; data not shown). To verify that guanylate cyclase is acting as an ANP-receptor, we used <sup>125</sup>I-labelled ANP in cross-linking experiments (Fig. 3). Transfected cells were incubated with <sup>125</sup>I-labelled ANP with or without excess unlabelled ANP. After cross-linking and SDS-polyacrylamide gel electrophoresis under reducing conditions, a single band migrating with *M<sub>r</sub>* 130K was evident from

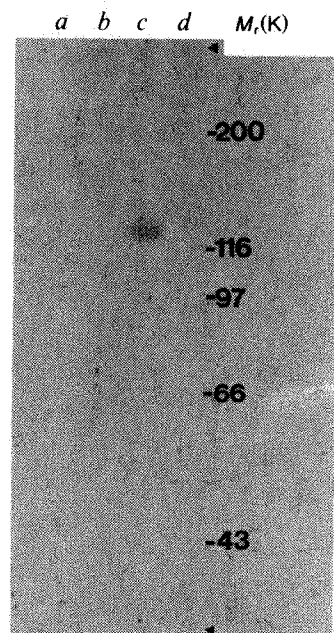


FIG. 3 Cross-linking of <sup>125</sup>I-labelled ANP to transfected COS-7 cells. Transfections are described in the legend to Table 1. Binding and cross-linking have been described previously<sup>15</sup>. Cells were washed 3 times with 2 ml Hanks' balanced salt solution (HBSS), then incubated for 1 h at room temperature in 1 ml HBSS containing 0.5 nM <sup>125</sup>I-labelled ANP (2,200 Ci mmol<sup>-1</sup>) in the absence (a, c) or presence (b, d) of 0.5 μM unlabelled ANP. 1 ml HBSS containing 2 mM disuccinimidyl suberate was added to a final concentration 1 mM and the incubation continued for 30 min. Cells were then washed with 2 ml HBSS and solubilized in 400 μl hot (95 °C) Laemmli sample buffer<sup>28</sup>, of which 150 μl was heated at 95 °C in the presence of 7.5 μl 2-mercaptoethanol and analysed by electrophoresis on a 7.5% SDS-polyacrylamide gel<sup>29</sup>, followed by drying and autoradiography. Positions of molecular weight markers are shown on the right; the top and bottom of the separating gel are indicated by arrowheads. a, b, Cells transfected with pSVL; c, d, cells transfected with pSVL-GC.

the cells transfected with pSVL-GC. This band was not seen in the presence of an excess of non-radioactive ANP or in cells transfected with pSVL. The mobility of this radioactive band coincides with that previously reported for the high-molecular weight ANP receptor<sup>12,15,23,24</sup>. Thus the guanylate cyclase cDNA encodes a protein having both guanylate cyclase and ANP-binding activities.

In conclusion, we have isolated and sequenced a cDNA clone for the membrane form of guanylate cyclase from rat brain. From the cDNA sequence we predict a protein divided by a single transmembrane domain into an amino-terminal extracellular ANP-binding domain, and a carboxyl-terminal intracellular catalytic domain (Fig. 4). The intracellular domain is homologous both with protein kinases and to a subunit of the soluble form of guanylate cyclase, whereas the extracellular domain is homologous with a low-molecular weight ANP receptor. Expression of the cDNA produces a protein with both ANP-binding and guanylate cyclase activity.

Previous cross-linking experiments indicated that the membrane form of guanylate cyclase in *Arbacia punctulata* could function as a receptor for egg-associated peptides which activate sperm respiration and motility<sup>25</sup>; one of the earliest effects of these egg peptides on spermatozoa is the stimulation of cyclic GMP synthesis<sup>26,27</sup>. But functional expression of the cloned sea urchin enzyme has not yet been obtained in mammalian cells or in *Xenopus* oocytes<sup>6</sup>, apparently because of a lack of proper post-translational modification. It has not been possible, therefore, to test directly whether the enzyme serves as a receptor. Several groups have also reported the co-purification of high-molecular weight ANP receptors with active guanylate cyclase<sup>12,13,24</sup>, but it is not clear whether the two activities reside within the same molecule<sup>12</sup>. Here we have demonstrated directly that the membrane form of guanylate cyclase functions as a receptor. The ANP receptor/guanylate cyclase we describe may represent, by analogy with the growth factor receptor/protein

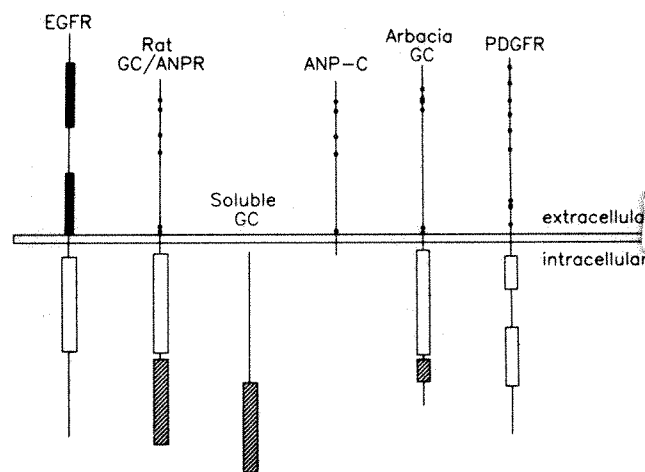


FIG. 4 Topology of members of the guanylate cyclase/ANP receptor family and the growth factor receptor/protein tyrosine kinase family. Drawn to scale are representations of the epidermal growth factor receptor (EGFR)<sup>30</sup>, the membrane forms of guanylate cyclase from rat (rat GC/ANPR) and from the sea urchin *Arbacia punctulata* (Arbacia GC)<sup>6</sup>, a subunit of the soluble form of guanylate cyclase (soluble GC)<sup>19</sup>, the ANP-C receptor (ANP-C)<sup>16</sup>, and the platelet-derived growth factor receptor (PDGFR)<sup>17</sup>. Drawing modelled after ref. 17. Individual cysteine residues are indicated by black circles in the extracellular domains of each protein, except in the case of the EGF receptor, in which the cysteine-rich domains are indicated by black boxes. Protein kinase domains (EGFR and PDGFR) and protein kinase-like domains (rat GC/ANPR, Arbacia GC) are indicated by open boxes. Areas of sequence similarity between the soluble and membrane forms of guanylate cyclase are indicated by hatched boxes.

tyrosine kinases, a member of a diverse family of cell-surface receptors. This family is unique with regard to the regulation of cyclic nucleotides, in that cGMP is formed directly by the receptor protein in response to the binding of an agonist. □

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## Molecular cloning and characterization of an insulin-regulatable glucose transporter

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A MAJOR mechanism by which insulin stimulates glucose transport in muscle and fat is the translocation of glucose transporters from an intracellular membrane pool to the cell surface<sup>1–5</sup>. The existence of a distinct insulin-regulatable glucose transporter was suggested by the poor cross-reactivity between antibodies specific for either the HepG2 or rat brain glucose transporters and the rat adipocyte glucose transporter<sup>6,7</sup>. More direct evidence was provided by the production of a monoclonal antibody (mAb 1F8) specific for the rat adipocyte glucose transporter that immunolabels a species of relative molecular mass 43,000 (43K) present only in tissues that exhibit insulin-dependent glucose transport<sup>8</sup>, suggesting that this protein may be encoded by a different gene from the previously described mammalian glucose transporters<sup>9–13</sup>. This antibody has been used to immunoprecipitate a 43K protein that was photoaffinity-labelled with cytochalasin B in a glucose displaceable way, and to immunolabel a protein in the plasma membrane of rat adipocytes, whose concentration was increased at least fivefold after cellular insulin exposure. Here we describe the cloning and sequencing of cDNAs isolated from both rat adipocyte and heart libraries that encode a protein recognized by mAb 1F8, and which has 65% sequence identity to the human HepG2 glucose transporter<sup>9</sup>. This cDNA hybridizes to an mRNA present only in skeletal muscle, heart and adipose tissue. Our data indicate that this cDNA encodes a membrane protein with the characteristics of the translocatable glucose transporter expressed in insulin-responsive tissues.

Several clones were isolated from a rat adipocyte and a rat heart cDNA library by low stringency hybridization using a fragment of the HepG2 glucose transporter cDNA as the probe. A 2,086-base pair (bp) consensus sequence (the 'IRGT sequence') compiled from four independent clones obtained from both libraries is shown in Fig. 1a. This sequence includes the complete coding region for a protein that shares sequence similarity with the HepG2 glucose transporter (Fig. 1b). The initiation codon was assigned to the first ATG triplet (beginning at position 154) downstream of an in-frame termination codon (TGA) beginning at position 133. An open reading frame extends

from this ATG triplet to position 1,680 that codes for a 509-amino acid polypeptide with a predicted relative molecular mass ( $M_r$ ) of 54,860. This value is in reasonable agreement with the estimated size (38K) of the deglycosylated transporter immunolabelled with mAb 1F8 (data not shown). The difference between the predicted and measured  $M_r$  values is similar to the difference between these values for the HepG2 glucose transporter, consistent with the anomalous migration of membrane proteins during SDS-PAGE<sup>9</sup>. Several clones were isolated from the adipocyte library that contained deletions in the 5' region of the mRNA compared to the IRGT sequence. The deleted regions were flanked by extremely GC-rich sequences. Thus, the deletions probably resulted from excision of hairpin loops formed during construction of the library.

The deduced amino-acid sequence of IRGT is 65.2% identical to that of the HepG2 glucose transporter (Fig. 1b). The sequence identity between the two proteins is particularly strong within the predicted transmembrane segments, with the notable exception of segment 3 (amino-acid residues 112–132 in the IRGT sequence, Fig. 1). Interestingly, there is little sequence homology between the IRGT (Fig. 1a), the HepG2 glucose transporter<sup>9</sup> and the liver glucose transporter<sup>12,13</sup> within this particular domain. There are considerable differences in the sequences of the HepG2 glucose transporter and IRGT within the predicted soluble domains of the HepG2 transporter<sup>9</sup>, particularly those encompassing the N and C terminus and the large, central, cytoplasmic loop. IRGT shares the putative glycosylation site (Asn 57) present in the HepG2 transporter (Asn 45) within a proposed exofacial loop after the first transmembrane segment. IRGT also contains the putative photoaffinity-labelling cytochalasin B-binding site (Trp 428) corresponding to Trp 412 in the HepG2 sequence<sup>14,15</sup>. The hydropathy plot of the IRGT protein sequence, deduced using the method of Kyte and Doolittle<sup>16</sup>, is nearly superimposable on that of the HepG2 glucose transporter<sup>9</sup> (data not shown), suggesting that these two proteins have very similar two-dimensional topologies with respect to the membrane. IRGT has slightly larger N-terminal, C-terminal and glycosylated loop domains compared to the HepG2 glucose transporter however.

RNA was transcribed from a 2,086-bp IRGT cDNA construction and translated in an *in vitro* rabbit reticulocyte lysate system. The primary translation product synthesized in the absence of microsomes had a slightly reduced mobility on SDS-polyacrylamide gels (apparent  $M_r$  = 39K) relative to the HepG2 translation product (apparent  $M_r$  = 37K), in reasonable agreement with the predicted difference in the relative molecular masses of the two proteins. Addition of dog pancreatic microsomes to the translation system decreased the mobility of both the IRGT (apparent  $M_r$  = 43K) and HepG2 (apparent  $M_r$  = 40K) proteins (Fig. 2) consistent with core glycosylation upon cotranslational

## *b*

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FIG. 1 a, Nucleotide sequence of IRGT cDNA and the deduced amino-acid sequence of the insulin-regulatable glucose transporter. The nucleotide sequence presented was compiled from the four clones whose sequences were identical in the overlapping regions. The two adipocyte clones encompassed residues 1–1,450 and 241–2,086. The two heart clones encompassed residues 141–1,212 and 717–1,944. Amino-acid residues are numbered above the sequence and nucleotides are numbered below. b, Deduced amino-acid sequences of the HepG2 glucose transporter and IRGT are aligned for comparison. The amino acids are indicated in the one-letter code. Regions of identity between the HepG2 glucose transporter and IRGT amino-acid sequences are indicated by a dash. Gaps have been introduced to optimize the alignment.

**METHODS.** Phage plaques ( $2 \times 10^6$ ) from both rat adipocyte and rat heart cDNA libraries in  $\lambda$ gt11 were screened under low stringency hybridization conditions (39 °C in 50% formamide,  $5 \times$  SSPE, 0.1% SDS,  $5 \times$  Dehnhardt's 250  $\mu$ g ml<sup>-1</sup> salmon sperm DNA) using a <sup>32</sup>P-labelled EcoRI fragment (from pGT25S ref. 9) of the HepG2 glucose transporter. Filters were washed at room temperature in  $1 \times$  SSC, 0.5% SDS and then at 42 °C in  $0.1 \times$  SSC, 0.5% SDS. Positive clones were plaque-purified and the cDNA inserts were sized after EcoRI digestion of the mini-prep DNA. Four clones (two from each library) were selected for further characterization. The inserts were subcloned into the EcoRI sites of M13mp18 and mp19 and were sequenced in both strands according to a modified version<sup>24</sup> of the Sanger method<sup>25</sup>. The sequence data were compiled by the computer-assisted method of Staden<sup>26</sup>. The insert DNAs were sequenced completely.

insertion of the protein into microsomes. Subsequent digestion with endoglycosidase H indicated that IRGT and the HepG2 glucose transporter were core-glycosylated to the same extent during insertion into microsomes (data not shown). The apparent  $M_r$  of the core glycosylated translation product of IRGT (Fig. 2) was similar to the  $M_r$  of the protein in fat and muscle immunolabelled by mAb 1F8 (Fig. 3). The IRGT translation product, but not that of the HepG2 glucose transporter RNA, was specifically immunoprecipitated using mAb 1F8 (Fig. 2). A polyclonal antibody (R820) was prepared by immunizing rabbits with a peptide corresponding to residues 498–509 within the predicted sequence of IRGT (see Fig. 1a). This antiserum also specifically immunoprecipitated the IRGT translation product (Fig. 2). Polyclonal and monoclonal antibodies raised against the human erythrocyte glucose transporter immunoprecipitated the HepG2 glucose transporter translation product but not that of IRGT (data not shown).

In contrast to the tissue distribution of the HepG2 glucose transporter<sup>17</sup> or human liver-like glucose transporter<sup>10</sup> mRNAs, IRGT mRNA was detected at relatively high levels in brown and white adipose tissue, heart and red and white skeletal muscle (Fig. 3a). There was no detectable hybridization to brain, liver, or HepG2 RNA. Rat kidney RNA had a weak signal, presumably due to contamination with renal brown fat during RNA purification. An RNA transcript was also present in 3T3-L1 adipocytes but not in pre-adipocytes (Fig. 3a). Qualitatively, the tissue distribution of IRGT mRNA was identical to the distribution of the protein as determined by immunoblotting with mAb 1F8 (Fig. 3c) or the peptide-specific antiserum, R820 (Fig. 3d). In addition, both mAb 1F8 (Fig. 4b) and R820 (Fig. 4a) immunolabelled a protein of about 45K in 3T3-L1 adipocytes but not in 3T3-L1 fibroblasts. The differences in tissue abundance of the 43K protein immunolabelled with these antisera (brown adipose tissue > heart > red muscle > white adipose tissue and white muscle) parallel the heterogeneity in *in vivo* insulin-stimulated glucose utilization rates that are observed among these tissues<sup>18–20</sup>. In contrast, using an anti-human erythrocyte glucose transporter antibody, the highest labelling among rat tissues was found in brain (Fig. 3b) and a similar concentration of this HepG2-like transporter was detected in both 3T3-L1 fibroblasts and adipocytes (Fig. 4b). Relatively weak immunolabelling was observed with the anti-human erythrocyte glucose transporter antibody in muscle, heart and fat. Interestingly, of six heart clones and seven adipocyte clones

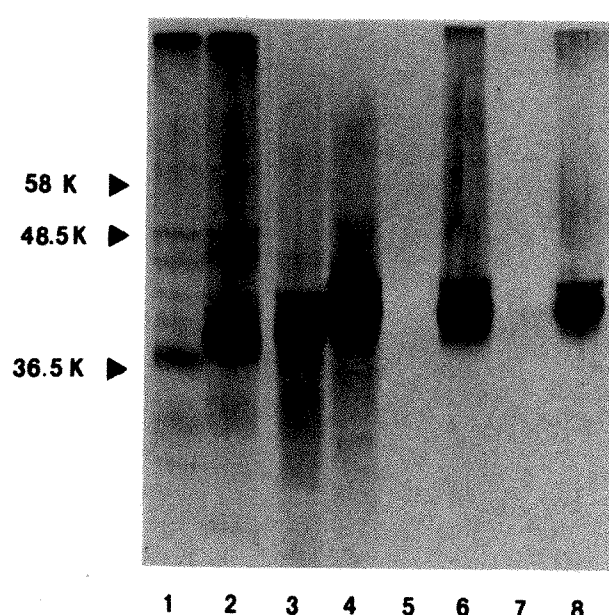


FIG. 2 Translation of IRGT RNA in a reticulocyte cell-free system. A 2,086-bp IRGT cDNA was constructed by ligation of unique EcoRI-ScaI fragments, derived from the two adipocyte clones described in the Fig. 1 legend, at the ScaI site. The resulting construct was subcloned into the EcoRI site of Bluescribe plasmid. IRGT and HepG2 GT mRNA<sup>27</sup> were synthesized *in vitro* using T7 or SP6 RNA polymerase, respectively, and then translated in a reticulocyte lysate system in the presence of <sup>35</sup>S-methionine as previously described<sup>27</sup>. Lysates or pelleted rough microsomes were analysed by SDS-PAGE using an 8% resolving gel. Lane 1, 25  $\mu$ l of lysate with HepG2 glucose transporter mRNA; lane 2, 25  $\mu$ l of lysate with IRGT mRNA; lane 3, 25  $\mu$ l of lysate with HepG2 glucose transporter mRNA translated in the presence of rough microsomes; lane 4, 25  $\mu$ l of lysate with IRGT mRNA translated in the presence of rough microsomes. IRGT RNA was translated in the presence of dog pancreatic microsomes, the microsomes were pelleted, washed, solubilized with 1% SDS and diluted (1:10, v:v) with PBS containing 1% Triton X-100 and then divided into two equal aliquots: immunoprecipitation using mAb 1F8 (ref. lane 6), immunoprecipitation using R820 antiserum (lane 8). HepG2 glucose transporter RNA was translated and immunoabsorbed using mAb 1F8 (lane 5) or R820 (lane 7). The relative migration of relative molecular mass standards is indicated on the left.

that were isolated using the HepG2 glucose transporter cDNA, none corresponded to the HepG2-type glucose transporter<sup>9,10</sup>. Thus, the HepG2-type transporter is probably expressed at low levels, if at all, in insulin-regulatable tissues. The emergence of the IRGT mRNA and protein after differentiation of 3T3-L1 fibroblasts into adipocytes is consistent with the induction of a highly sensitive and responsive hexose transport system under these conditions<sup>21,22</sup>. These data indicate that 3T3-L1 cells may provide a unique model for studying the factors involved in regulating expression of the insulin-regulatable glucose transporter as well as the cellular factors that confer tissue-specific insulin-dependent glucose transport.

Further evidence that IRGT encodes an insulin-regulatable protein is shown in Fig. 4a. The R820 antiserum immunolabelled a protein of similar size to that reported for the adipocyte glucose transporter in the low-density microsomes of untreated rat adipocytes (reviewed in ref. 8). Consistent with previous data obtained using mAb 1F8 (ref. 8), R820 immunolabelled a 43K protein in a plasma membrane fraction from rat adipocytes, the concentration of which was increased about sevenfold upon cellular insulin exposure (Fig. 4a). Similar results have been observed with 3T3-L1 adipocytes (data not shown). These data provide further support for the glucose transporter translocation hypothesis<sup>1,2</sup>. The competition data presented in Fig. 4c indicate

that the epitopes recognized by mAb 1F8 and R820 either overlap or are identical, that the epitope of mAb 1F8 is specific for the C terminus of the protein, and that mAb 1F8 and R820 do recognize the same protein in adipocyte membrane fractions.

Several strands of evidence suggest that IRGT encodes the acutely insulin-responsive glucose transporter: (1) the IRGT protein shares considerable sequence and structural identity with other glucose transporters<sup>9,10,13</sup>, (2) mAb 1F8 immuno-

precipitates the IRGT protein; (3) the tissue distribution of the IRGT protein parallels the acute insulin responsiveness with respect to glucose transport of various tissues *in vivo*; (4) insulin stimulates the translocation of the IRGT protein from a low-density microsomal fraction to the plasma membrane in rat and 3T3-L1 adipocytes; and (5) the differentiation of 3T3-L1 fibroblasts into adipocytes is accompanied by the appearance of IRGT mRNA and protein. Definitive evidence that the cDN/ clone described in this study encodes a protein that transport

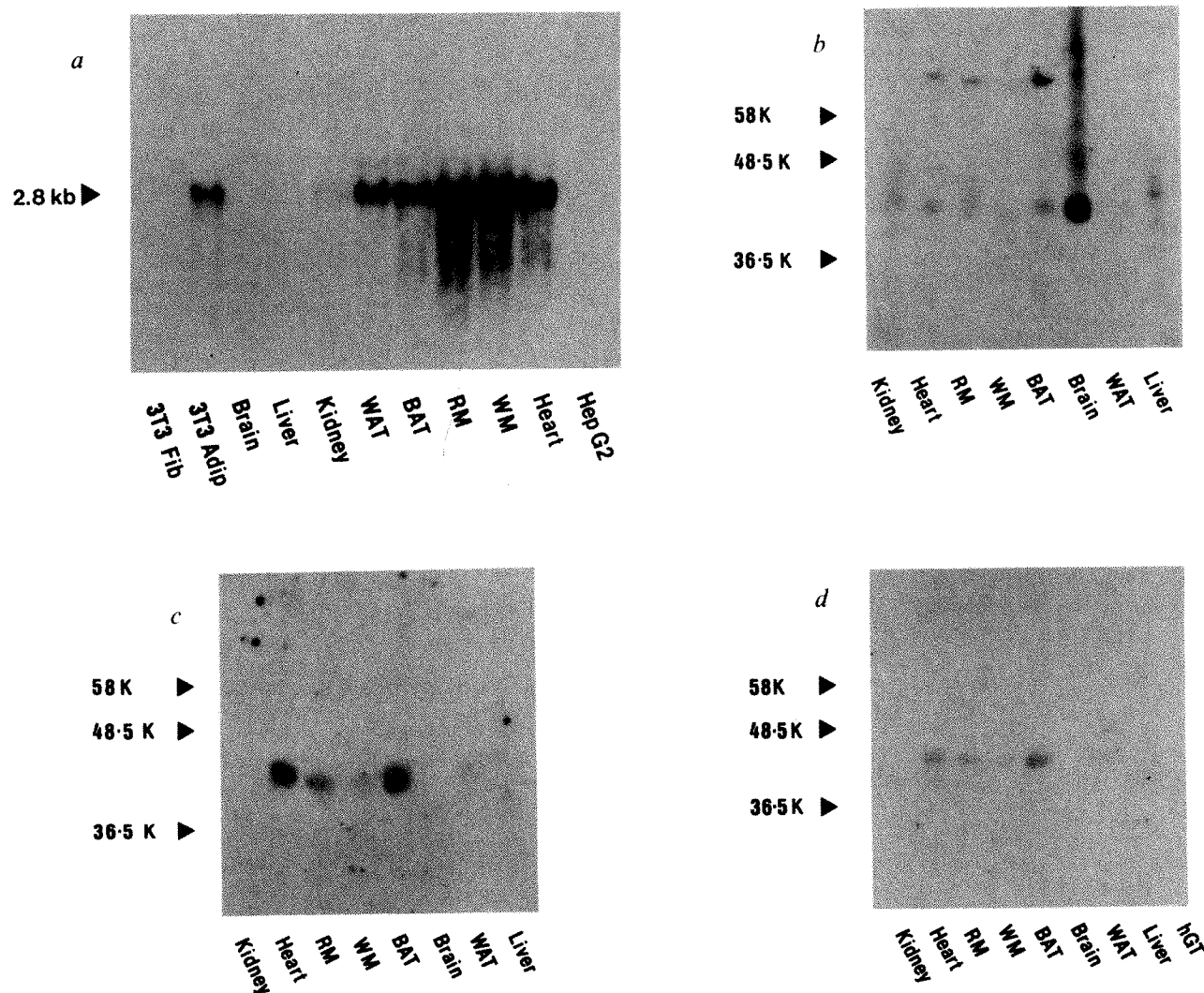
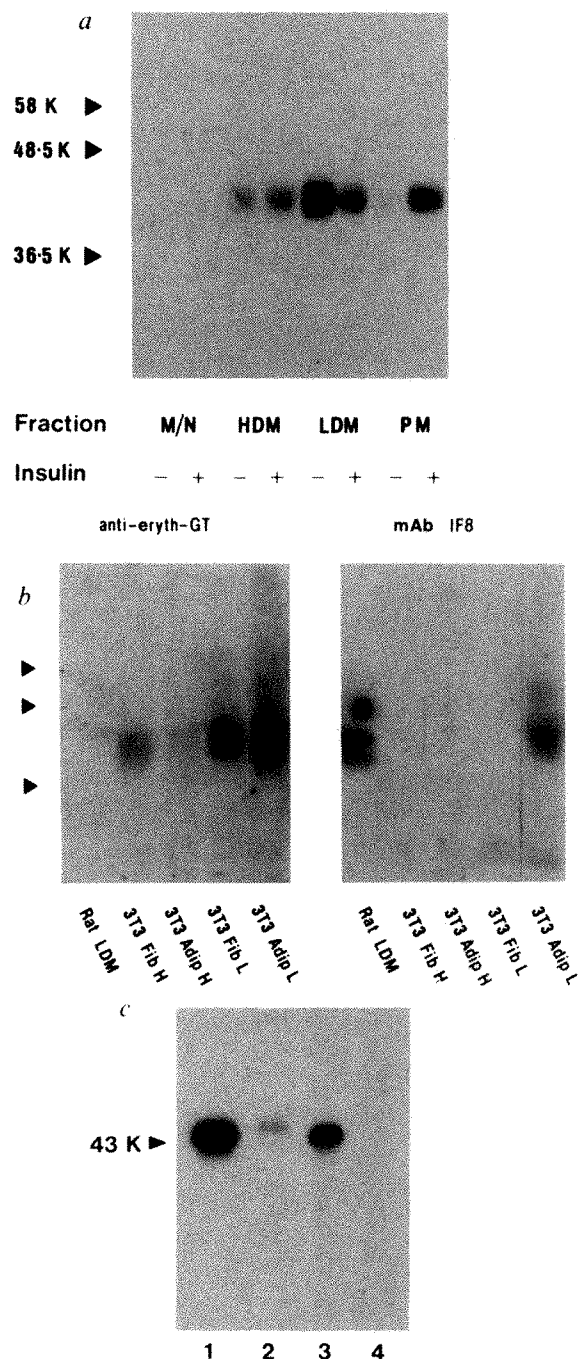


FIG. 3 Tissue distribution of IRGT mRNA and protein. *a*, Total RNA isolated from various rat tissues probed with IRGT cDNA. Abbreviations: 3T3-L1 fibroblasts, Fib; 3T3-L1 adipocytes, Adip; rat white adipose tissue, WAT; rat brown adipose tissue, BAT; rat red skeletal muscle, RM; rat white skeletal muscle, WM. *b*, Rat tissues were homogenized in PBS containing 1% Triton X-100. Total tissue protein (100 µg) from rat tissues were analysed by SDS-PAGE followed by immunoblotting using a polyclonal antisera against the human erythrocyte glucose transporter<sup>9</sup>. Immunoreactive glucose transporter was visualized using <sup>125</sup>I-labelled Protein A (Amersham). *c*, Total protein extracts (100 µg) from rat tissues were immunoblotted using mAb 1F8. Immunolabelled bands were visualized using <sup>125</sup>I-labelled sheep anti-mouse IgG (Amersham). *d*, Total protein extracts (100 µg) from rat tissues and 1 µg of purified human erythrocyte glucose transporter (hGT) were immunoblotted using a polyclonal antisera (R820) specific for a peptide derived from the C-terminal sequence of IRGT. Iodo-labelled Protein A was used to visualize the immunolabelled proteins.

**METHODS.** Total RNA was isolated (*a*) from rat tissues using the guanidinium, CsCl procedure<sup>28</sup>. The samples (50 µg of each) were electrophoresed on a

formaldehyde-agarose gel (1.2%) and then blotted and fixed onto a nylon membrane. The blot was hybridized to IRGT cDNA labelled with <sup>32</sup>P by nick-translation, washed, and exposed to Kodak XAR-5 film as previously described<sup>19</sup>. RNA markers are in an adjacent lane. For *d*, a 12-amino-acid peptide (Thr-Glu-Leu-Glu-Tyr-Leu-Gly-Pro-Asp-Glu-Asn-Asp), corresponding to the 12 terminal amino acids within the C terminus of IRGT (Fig. 1a) was synthesized by the Protein Chemistry laboratory at Washington University School of Medicine. A cysteine was included at the N terminus of the peptide to facilitate coupling of 12.5 mg of the peptide to keyhole limpet haemocyanin (KLH) (40 mg), using the cross-linking reagent *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Pierce, Rockford, Illinois), as previously described<sup>29</sup>. The peptide-KLH conjugate was emulsified in Freund's complete adjuvant and injected at multiple sites intradermally (500 µg) into three New Zealand white rabbits. The rabbits were boosted at three-week intervals with the peptide conjugate (300 µg) emulsified in incomplete adjuvant and bleeds were obtained two weeks after this boost. Sera obtained from all three rabbits tested positive by immunoblotting rat adipocyte subcellular fractions. One rabbit serum (R820) was selected for further study as described here.



**FIG. 4** IRGT encodes an insulin-regulatable protein. *a*, Immunoblot using polyclonal antisera (R820) of subcellular membrane fractions (50  $\mu$ g) obtained from rat adipocytes after incubation in the absence or presence of insulin ( $10^{-8}$  M) for 15 min: mitochondria/nuclei (M/N), high-density microsomes (HDM), low-density microsomes (LDM) and plasma membranes (PM). *b*, Immunoblot of 3T3-L1 fibroblasts and 3T3-L1 adipocyte membrane fractions (100  $\mu$ g) using either a polyclonal antibody specific for the human erythrocyte glucose transporter (anti-eryth GT)<sup>9</sup> or mAb 1F8. Confluent plates of either 3T3-L1 fibroblasts or adipocytes were homogenized in a buffer containing 20 mM HEPES, 250 mM sucrose, 1 mM EDTA, pH 7.4. Total membrane pellets were layered onto a 1.12 M sucrose cushion, and centrifuged at 100,000*g* in an SW 41 rotor (Beckman). This procedure resolved membrane fractions above (L) and below (H) the sucrose cushion, of similar protein concentration for fibroblasts and adipocytes. Rat LDM (50  $\mu$ g) was electrophoresed in an adjacent lane for comparison. *c*, Rat adipocyte low-density microsomes (20  $\mu$ g) were immunoblotted with a polyclonal antisera, R820, at a dilution of 1:500 (lane 1), or with R820 (1:500) in the presence of mAb1F8 (20  $\mu$ g ml<sup>-1</sup>, lane 2), with mAb1F8 alone (2  $\mu$ g ml<sup>-1</sup>, lane 3) or with mAb1F8 (2  $\mu$ g ml<sup>-1</sup>) in the presence of R820 (1:20, lane 4). Immunoreactive species were detected using <sup>125</sup>I-labelled Protein A (lanes 1 and 2) or <sup>125</sup>I-labelled sheep anti-mouse IgG (lanes 3 and 4).

glucose will require functional expression. It is interesting that several bacterial proton symporters that do not seem to transport glucose also have significant homology with the HepG2 glucose transporter<sup>23</sup>. Thus, it is conceivable that IRGT represents a member of this gene family, whose function involves the transport of a substrate other than glucose. In any case, the overall phenotype of IRGT suggests that this protein has an important role in insulin action. A gene encoding an insulin-regulatable glucose transporter that is expressed only in muscle and fat would be an obvious target for a genetic defect causing predisposition toward insulin-resistant states such as non-insulin-dependent diabetes mellitus. □

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## Double-strand breaks at an initiation site for meiotic gene conversion

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It has been proposed that the initiation of meiotic recombination involves either single-strand or double-strand breaks in DNA<sup>1-3</sup>. It is difficult to distinguish between these on the basis of genetic evidence because they give rise to similar predictions<sup>4-6</sup>. All models invoke initiation at specific sites to explain polarity, which is a gradient in gene conversion frequency from one end of a gene to the other. In the accompanying paper<sup>7</sup> we describe the localization of an initiation site for gene conversion to the promoter region of the *ARG4* gene of the yeast *Saccharomyces cerevisiae*. Here, we show that a double-strand break appears at the *ARG4* recombination initiation site at the time of recombination, and that the broken DNA molecules end in long single-stranded tails.



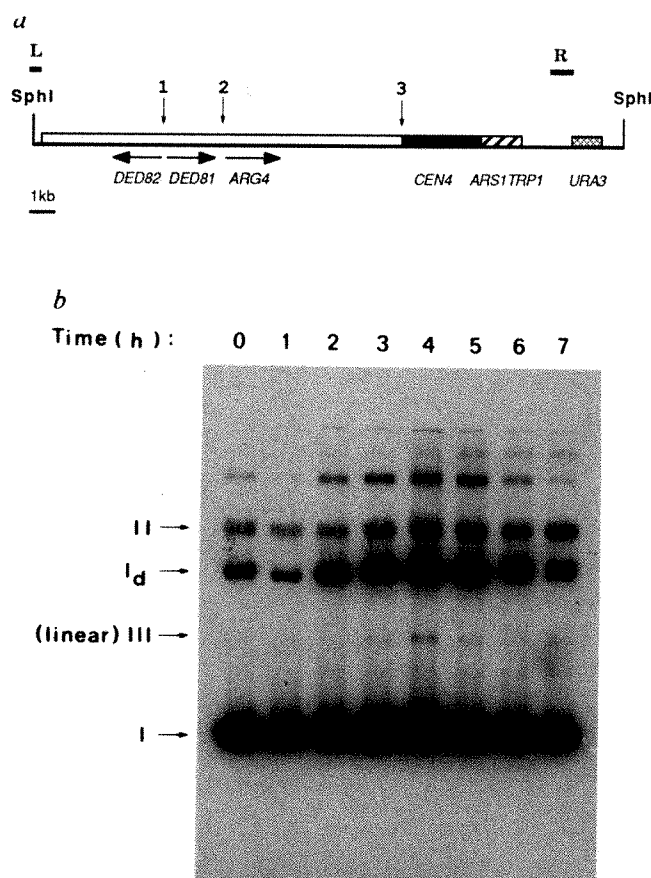


FIG. 1 *a*, Map of plasmid p(SPO13)2 (ref. 27) linearized with *Sph*I. The location of the centromere and yeast genes are shown under it. The three meiosis-specific cleavage sites are indicated by arrows. *R* and *L* represent probes used to detect cleaved fragments. The open box represents 15 kb of DNA from the yeast *ARG4* locus. *b*, Time course of undigested plasmid DNA through meiosis. The lowest, most intense band (I) represents supercoiled monomers, band *I<sub>d</sub>* is supercoiled dimer circles, band II is relaxed monomer circles and the transient band III is due to linearized plasmid molecules. The identity of the bands was established by two-dimensional electrophoresis<sup>28</sup> and comparison with known markers.

**METHODS.** Cells of strain NKY278 (*MATa/MATα*, *LYS2::HO/lys2::HO*, *lys2/lys2*, *ura3/ura3*) carrying plasmid p(SPO13)2 were grown mitotically in YP-acetate medium (1% yeast extract, 2% bacto-peptone, 1% potassium acetate) and transferred to sporulation medium (1% potassium acetate) to start meiosis. Aliquots were removed every hour, frozen, and DNA prepared as described<sup>29</sup>. The DNA was analysed by electrophoresis on a 0.4% agarose gel, transferred to Nytran membrane and detected with a <sup>32</sup>P-labelled pBR322 (vector-specific) probe.

We have examined the DNA of a yeast plasmid containing a meiotic recombination initiation site for changes related to recombination as the cell progresses through meiosis. The plasmid carries a 15-kilobase (kb) fragment of DNA that includes the *ARG4* gene (Fig. 1*a*). The diploid yeast strain we used is a derivative of SK-1 (ref. 8), which undergoes a rapid and synchronous meiosis upon transfer to sporulation medium.

Total DNA was extracted from yeast cells collected at hourly intervals after transfer to sporulation medium and the structure of the plasmid DNA was analysed by Southern blotting<sup>9</sup>. Figure 1*b* shows that the plasmid exists predominantly as supercoiled monomers immediately after transfer to sporulation medium, with some relaxed monomer and various dimer forms. All of these persist through meiosis. As meiosis proceeds however, a new band appears with the mobility of full-length linear plasmid DNA. This band is visible three hours after transfer to sporulation medium, peaks in intensity at four hours, and has decreased

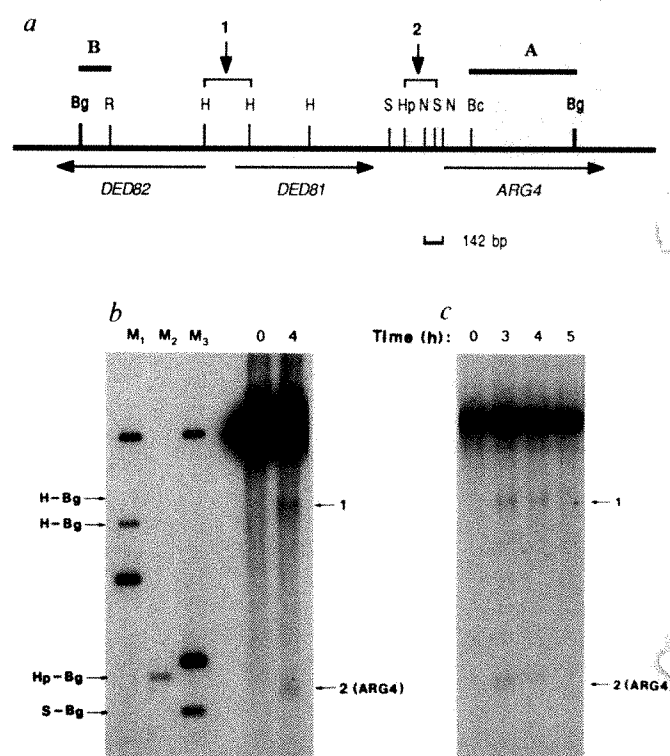


FIG. 2 *a*, Partial restriction map of the *ARG4* region. Restriction sites in and near *ARG4* and two adjacent essential genes, *DED81* and *DED82*, represented by arrows covering their open reading frames. *A* and *B* represent restriction fragments used as probes. *Bc*, *Bc1*, *Bg*, *Bg1*, *R*, *EcoR*, *H*, *HindIII*, *Hp*, *HpaI*, *N*, *NspHI*, *S*, *SspI*. The 142-bp *NspHI* fragment which was deleted to remove the initiation site is shown below. *b*, Higher resolution mapping of meiosis-specific cleavage sites 1 and 2. DNA from mitotic cells (0 hours), and cells four hours into meiosis was cleaved with *Bgl*I, electrophoresed on a 0.7% agarose gel, blotted and probed with probe *A*. The labelled fragments in the marker lanes extend from the right-hand *Bgl*I site to the restriction sites indicated. Fragments labelled 1 and 2 are seen in the lane containing the 4-h sample but not the 0-h sample lane, and represent cleavage of the *Bgl*I fragment DNA at either site 1 or site 2 respectively. The strongly hybridizing band appearing in both the 0- and 4-h sample lanes represents intact 5-kb *Bgl*I fragment DNA. The *ARG4* cleavage site maps between the *Hpa*I and *Ssp*I restriction sites in the *ARG4* promoter region. The locations of cleavage sites 1 and 2 were confirmed by mapping from the other side of the break using probe *B* (data not shown). *c*, Analysis of chromosomal DNA from strain NKY278 before (0 h) and during (3, 4 and 5 h) meiosis. Cleavages at sites 1 and 2 are still visible, as represented by fragments 1 and 2.

in intensity by five hours. The linear DNA appears at the same time (2.5–5 h) as commitment to meiotic recombination occurs in SK-1 and its derivative strains<sup>10–11</sup>. In addition, we find that the products of recombination between two restriction site mutations in the *LEU2* gene<sup>12</sup> in an SK-1 strain background are first detectable at four hours, but do not reach their maximal level until five to six hours (data not shown).

We used restriction enzyme digestion to show that the linear plasmid molecules are generated by cleavage at specific sites and to map these sites. Our data indicate that there are three distinct subpopulations of linear plasmid molecules, each resulting from a double-strand break at one of three sites (1, 2 and 3 in Fig. 1*a*). Each subpopulation consists of about 0.5–1% of the total plasmid population, as determined from comparison with serial dilutions of genomic DNA.

More detailed mapping of the break sites (Fig. 2) revealed that site 2 is located within a 261-base pair (bp) region between the *Hpa*I and *Ssp*I sites immediately 5' to the *ARG4* coding region. Both the *ARG4* transcriptional promoter<sup>13</sup> and the

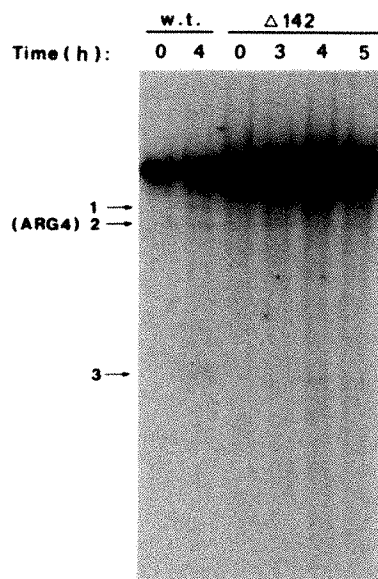


FIG. 3 Effect of deleting the *ARG4* initiation site on the meiosis-specific double-strand break. Plasmid p(SPO13)2- $\Delta$ 142 is identical to p(SPO13)2, except that 142-bp are deleted in the *ARG4* promoter region that removes the recombination initiation site<sup>7</sup>. DNA was prepared from cells containing either this plasmid or its wild-type parent (w.t.), before and during meiosis. Samples were digested with *Sph*I, electrophoresed on a 0.5% agarose gel and the fragments detected with probe R (Fig. 1a). The top intense band represents full-length (25 kb) linear plasmid. The three fainter bands are 19, 17 and 10 kb long, and arise from cleavage at sites 1, 2 and 3 respectively. The break in the *ARG4* promoter region (site 2) is absent in the deletion plasmid.

initiation site for meiotic gene conversion (see Fig. 2 in ref. 7) lie in this region. Break site 1 is located within a 450-bp region between two *Hind*III sites, which contains promoters for two divergently transcribed genes, *DED81* and *DED82* (ref. 7; N.P.S. and H.S., unpublished data). Site 3 maps near a functional promoter in the plasmid vector<sup>14</sup>.

Deletion of a 142-bp *Nsp*HI fragment in the *ARG4* promoter region decreases gene conversion within *ARG4* from 7.4% to 2% because it removes the *ARG4* initiation site (see ref. 7, Fig. 2, deletion  $\Delta$ 9). To determine if the same sequence is required for DNA breakage during meiosis, a plasmid derivative carrying the 142-bp deletion was studied in the same strain background. The two plasmids are compared in Fig. 3. The breaks at sites 1 and 3 are unaffected by the deletion and serve as internal controls. The break at site 2 however, is undetectable in the construct carrying the 142-bp deletion.

If the meiosis-specific double-strand breaks are relevant to normal meiotic recombination, they must occur on chromosomal DNA as well as on plasmid DNA. We analysed yeast chromosomal DNA in strain NKY278 in the absence of the plasmid. The breaks at sites 1 and 2 can be detected on chromosomal DNA at the same time interval after transfer to sporulation medium as is observed for plasmid DNA (compare Fig. 2b and c).

We probed the structure of the DNA termini by treating the DNA samples with S1 nuclease, which is specific for single-stranded DNA<sup>15</sup> (Fig. 4). The intensities of the fragments corresponding to breaks at sites 1, 2 and 3 remain constant after S1 nuclease treatment but, remarkably, the size of each fragment decreases. The change in fragment mobility can be seen after 2.5 min of S1 treatment and is complete at 10 min, when all these molecules have been shifted to a faster-migrating species. Prolonged S1 treatment does not result in further degradation of the fragments. The susceptibility of all the broken ends to S1

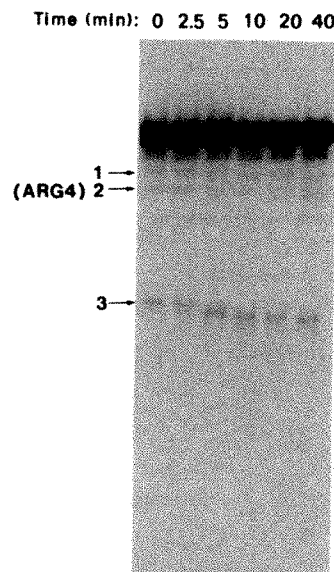


FIG. 4 Treatment of meiotic DNA samples with S1 nuclease. DNA from cells four hours into meiosis was treated with S1 nuclease in buffer containing 200 mM NaCl for the times indicated, digested with *Sph*I, and processed as in Fig. 3. The mobility of the full-length linear plasmid (25 kb) is unaffected by S1, whereas the cleaved fragments 1, 2 and 3 (19, 17 and 10 kb) show an initial rapid decrease in size, indicating that they all end in several hundred bases of single-stranded DNA.

nuclease indicates that all of these molecules terminate with a region of single-stranded DNA. The specificity of S1 nuclease means that this single-stranded region is probably degraded by the nuclease until the enzyme reaches a duplex region<sup>16</sup>. When we examined the termini corresponding to the other side of the break, we observed the same S1-sensitive profile (data not shown). Thus, both sides of each of the three breaks end with single-stranded DNA. A similar pattern of sensitivity to S1 nuclease was observed with DNA samples taken at 3, 4, or 5 h into meiosis (data not shown). The single-stranded tails are therefore seen as early as the breaks can be detected. The change in mobility of each of the fragments corresponds to a size difference of a few hundred nucleotides.

To confirm the presence of single-stranded tails on the cut molecules, we made use of the selective binding of single-stranded DNA to nitrocellulose membranes<sup>17</sup>. When restricted DNA is transferred directly from the gel without prior alkaline denaturation, fragments representing breaks at sites 1 and 2, are visible on the blot, whereas the intact fragment is absent (data not shown). Control experiments have shown that denatured, but not native, plasmid markers can be detected on these blots. Thus, non-denaturing Southern blots selectively enrich for the population of DNA molecules containing the broken ends, providing strong evidence for these molecules having single-stranded DNA at their termini.

Four arguments can be used to support the involvement of the DNA breaks we observe in the initiation of recombination. First, the DNA breaks are most abundant between three and five hours after transfer to sporulation medium, the time at which meiotic recombination occurs<sup>10,11</sup>. Second, these breaks occur at specific sites, one of which is the initiation site for meiotic gene conversion at *ARG4*<sup>7</sup>. Third, a deletion that removes the initiation site for meiotic gene conversion at *ARG4* eliminates or greatly diminishes the double-strand break at *ARG4*. Finally, both sides of each of the three breaks end in a few hundred nucleotides of single-stranded DNA. This is consistent with the invasion of duplex DNA by single-stranded DNA being a critical step in the initiation of recombination<sup>1-3</sup>.



Our data provide strong circumstantial evidence in favour of the double-strand break repair model, but do not eliminate alternative origins for the breaks. First, the double-strand breaks might be formed *in vitro* during DNA preparation. We see the double-strand breaks and their associated single-stranded tails when DNA is prepared by several different procedures, including a very rapid preparation in liquid nitrogen<sup>18</sup>. Also, S1 nuclease treatment does not increase the intensity of the fragments, as might be expected if they arose from partial breakage of a single-stranded lesion present *in vivo*. Alternatively, the breaks may arise *in vivo* as consequences of recombination. Holliday junction resolution could lead to duplexes containing nicks on both strands, or mismatch repair could proceed by double-strand gap repair<sup>19</sup>. These possibilities are unlikely because we see the breaks in homozygous diploids at the initiation site and as early as three hours into meiosis.

Assuming that the breaks we see are involved in initiation, it is likely that the single-stranded tails are involved in strand invasion, as proposed in the double-strand-break repair model for recombination<sup>2</sup>. Strand-exchange activities have recently been purified from vegetatively growing and meiotic *S. cerevisiae* cells<sup>20-21</sup>. The single-stranded DNA could therefore generate several hundred bases of heteroduplex DNA adjacent to the region of double-strand gap repair. Extensive heteroduplex DNA has been found adjacent to the ends of linear molecules in plasmid transformation systems<sup>22,23</sup>. The generation of extensive heteroduplex DNA could explain the occurrence of high levels of post-meiotic segregation for certain alleles of *ARG4*<sup>4,7,24</sup>.

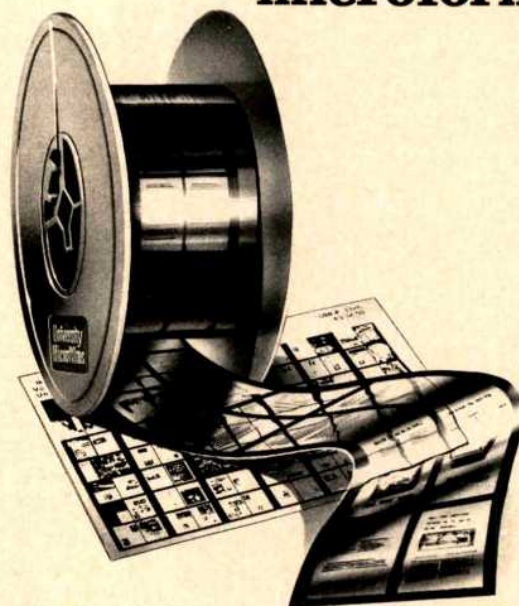
Two of the break sites map within transcriptional promoters, one at the *ARG4* promoter and one between the divergently transcribed genes *DED81* and *DED82*. The third is in the vicinity of a promoter that directs transcription towards the *CEN4* region of the YCp19 vector<sup>14,25</sup>. Are these promoters identical to the recombination initiation sites? A sequence that stimulates mitotic recombination, *HOT1*<sup>26</sup>, has recently been shown to be identical to the rDNA promoter. More detailed mapping of the *ARG4* initiation site may resolve the question of the relationship between transcription and recombination initiation. □

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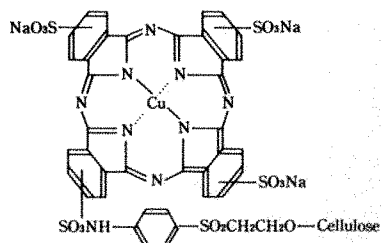
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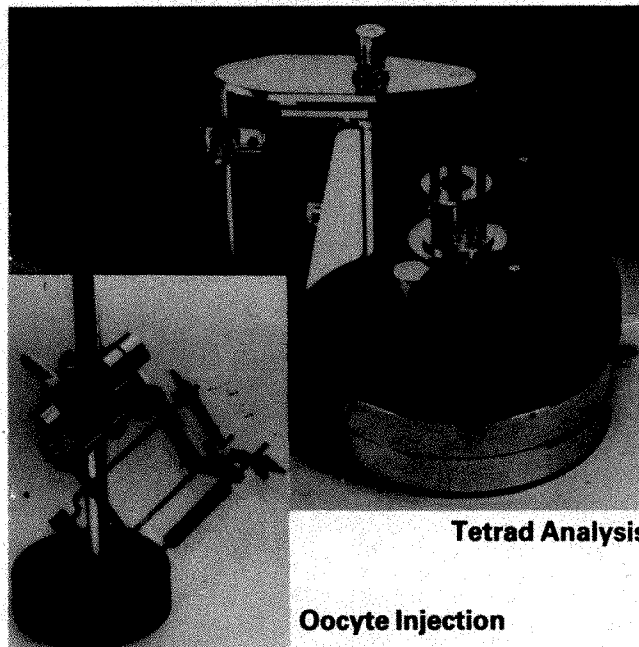
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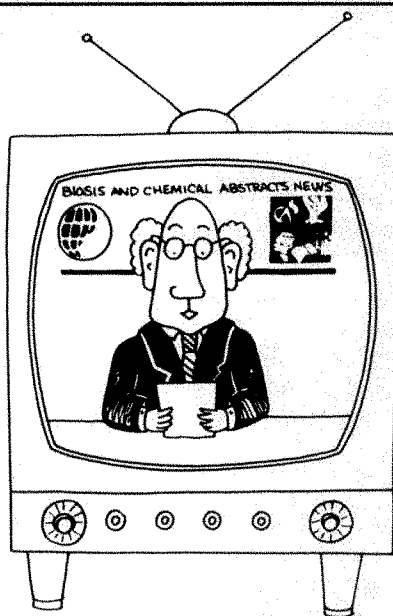
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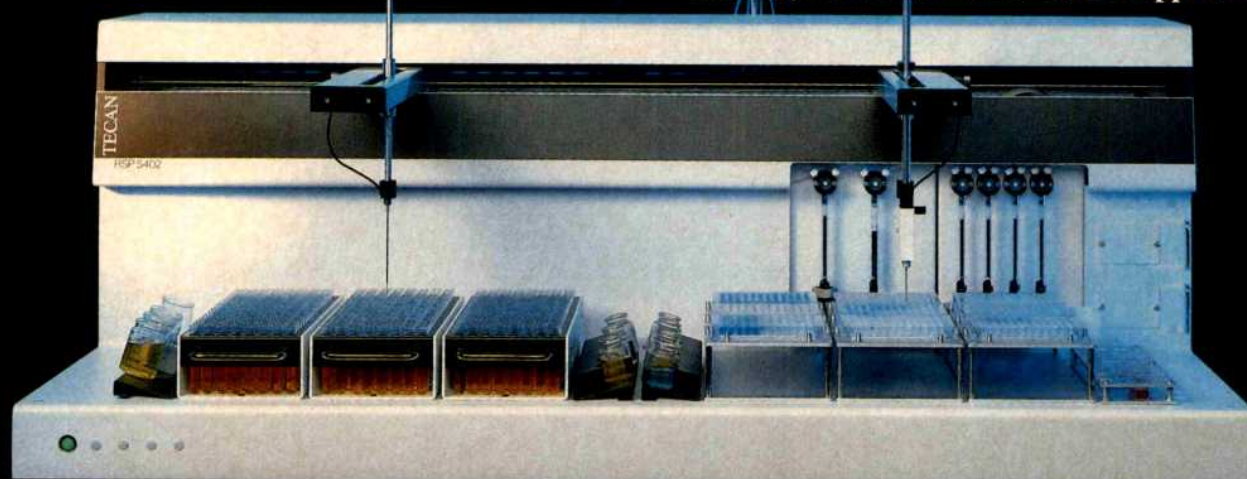
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# Countercurrent chromatography meets MS

Y.-W. Lee and R.D. Voyksner

Interfacing countercurrent chromatography with thermospray mass spectrometry provides a new analytical tool for detecting nonvolatile, hydrophilic or thermally unstable bioactive natural products.

THE development in the 1980s of modern countercurrent chromatography (CCC) instrumentation based upon the fundamental principles of liquid-liquid partition has caused a resurgence of interest in the separation sciences. The advantages of liquid-liquid extraction — the process for separating a multicomponent mixture according to its differential solubility in two immiscible solvents — have long been recognized. In spite of the limitations of the traditional countercurrent distribution methods which prevailed in the 1950s and 1960s, liquid-liquid extraction was used successfully to fractionate commercial insulin into two subfractions differing by only one amide group out of a molecular weight of 6,000 (ref. 1).

In recent years, significant improvements have been made to enhance the performance and efficiency of countercurrent methods<sup>2</sup>. The newly developed high-speed CCC technique utilizes a particular combination of coil orientation and planetary motion to produce a unique hydrodynamic phenomenon in the unilateral phase distribution of two immiscible solvents in a coiled column. The hydrodynamic properties can effectively be applied to perform a variety of countercurrent chromatographies, including true countercurrent<sup>3</sup> and foam countercurrent methods<sup>4</sup>. More recently, a 2,000 r.p.m. high-speed CCC has been developed which performs separations with speeds and resolutions comparable to those achieved using HPLC<sup>5</sup>.

We have successfully applied the high-speed CCC system in the separation of plant alkaloids, plant indole hormones, herbicides and bioactive lignins. One of the most distinct advantages offered by CCC is its lack of complications arising from solid supports, such as adsorptive sample loss, deactivation and contamination. Because the two-phase solvent system commonly used in CCC often consists of an organic phase and an aqueous phase, the technique also particularly lends itself to interfacing with mass spectrometry (MS). A solvent with a high aqueous percentage can be used with a volatile buffer such as ammonium acetate to achieve optimal conditions for thermospray MS without suffering the reduction in MS sensitivity typically observed in HPLC/MS with mobile phases low in water content<sup>6</sup>.

MS is generally regarded as the most versatile and universal detection tech-

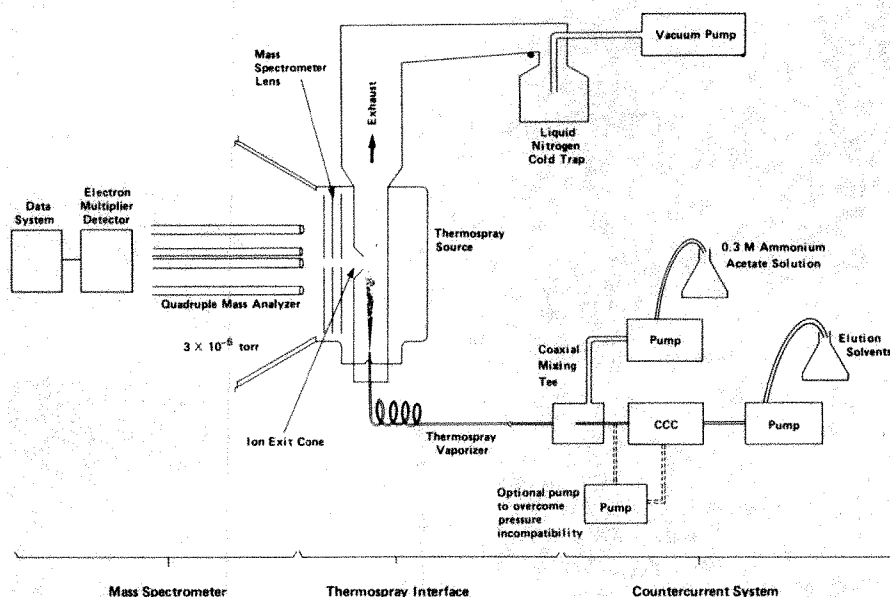


FIG. 1 Schematic diagram of the CCC/MS system.

nique for use with CCC because of its ability to detect compounds with no ultra-violet chromophore and provide precise identification of molecules. The pressure tolerance of high-speed CCC allows it to be interfaced to thermospray<sup>7</sup> mass spectrometry through an additional pump (to overcome thermospray backpressure). When the resolving power of CCC is coupled in real time with the sensitivity of MS, the individual chromatographic components need not be pure to acquire mass spectra sufficient for identification purposes. Reconstructed single-ion plots in which all of the ions of a given  $m/z$  value are plotted against retention time can be used to determine the purity of the total ion current chromatogram (TIC), where the mass spectrometer serves as a compound selective detector. If the intensity of the TIC peak does not rise and fall in accordance with the plot, it is not pure. The on-line coupling of CCC and MS also provides data on trace components which are not easily obtained when the techniques are performed separately.

## Configuration details

In our system, shown in Fig. 1, the effluent from a CCC operating at  $0.8 \text{ ml min}^{-1}$  is introduced into a pump through a zero dead volume tee fitted with a reservoir. Because the CCC produces varying flow rates, the additional pump is operated at  $0.7 \text{ ml min}^{-1}$ ; the reservoir provides extra solvent or vents excess solvent from the

CCC system as the pressure fluctuates. The effluent from the pump is mixed coaxially with  $0.3 \text{ M}$  ammonium acetate added at  $0.3 \text{ ml min}^{-1}$  to create a solvent with sufficient volatility for ion evaporation ionization<sup>8</sup>. The solvent is passed at a rate of  $1 \text{ ml min}^{-1}$  through a UV detector ( $254 \text{ nm}$ ) and into the thermospray interface. (At lower CCC flow rates of  $0.3\text{--}0.6 \text{ ml min}^{-1}$  the pressure drop across the thermospray vapourizer is sufficiently low to permit the direct coupling of the CCC effluent to the thermospray interface without the use of the additional pump.)

The thermospray interface connected to the quadrupole mass spectrometer is kept at a source temperature of  $250^\circ\text{C}$  and a vapourizer temperature of about  $170^\circ\text{C}$  to maximize the chromatography solvent cluster, which has been shown to co-maximize with the analyte under study<sup>7</sup>. No splitting of the CCC effluent is necessary. The solvent is pumped out of the source with a liquid nitrogen cold trap attached to a mechanical rough pump. The analysis is performed employing both negative and positive ion detection using ion evaporation ionization and chemical ionization.

## Early results

We have used the thermospray CCC/MS technique successfully in preliminary studies of a mixture of plant alkaloids<sup>9</sup>. Results obtained in our recent application of the technique to the study of bioactive



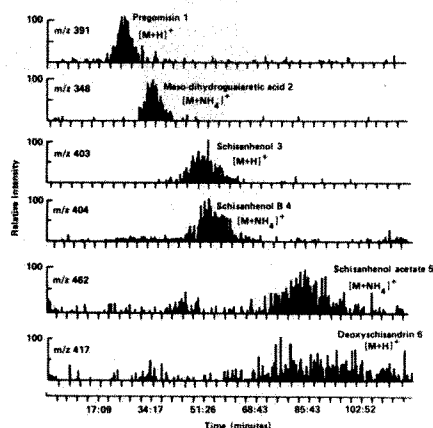


FIG. 2 CCC/MS ion chromatograms of the six major lignins from *Schisandra*. The chemical ionization filament in the quadrupole MS was operated at 1,000 V with a 0.15 mA emission current. The MS was scanned from  $m/z$  180 to  $m/z$  600 in 2 s. The mass calibration of the quadrupole was verified with polypropylene glycol (AMW = 1,000).

lignins<sup>10</sup> and triterpenoic acids<sup>11</sup> suggest that CCC/MS would be complementary to HPLC/MS as a useful method.

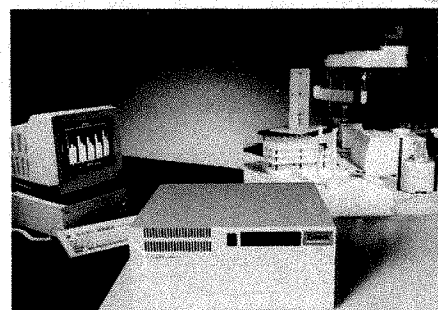
A CCC/UV chromatogram of a crude extract of the bioactive lignin from the herb *Schisandra* shows four distinct peaks. We have used thermospray CCC/MS to confirm the identification of the peaks and further to identify the minor components of the extract. The CCC/MS-selected ion chromatograms (Fig. 2) for the  $[M + H]^+$  or  $[M + NH_4]^+$  ion of the possible lignins from *Schisandra* indicated six components to be present. The major CCC/UV components present in the extract were confirmed to be schisanhenol and schisanhenol acetate based upon comparison of their thermospray spectra and retention times with the standards for these two components. The minor bioactive lignins such as schisanhenol B4 and deoxyshisandrin 6 were not sufficiently separated from schisanhenol and schisanhenol acetate by CCC with UV detection. But CCC/MS enabled the detection and acquisition of clean spectra of these trace components, which coeluted with the major components in the sample even though they exhibited different characteristic ions. The negative ion spectra for these lignins were 10 to 100 times less intense, and recorded only the major components.

The thermospray CCC/MS total ion current chromatogram presented a different picture of the relative abundances of the bioactive lignins than that obtained using CCC with UV detection. The thermospray CCC/MS response factors of the lignins are more similar than their UV extinction coefficients, enabling a more accurate estimate of the relative percentages of each lignin. Thermospray CCC/MS showed the major active lignins

## Pittsburgh preview

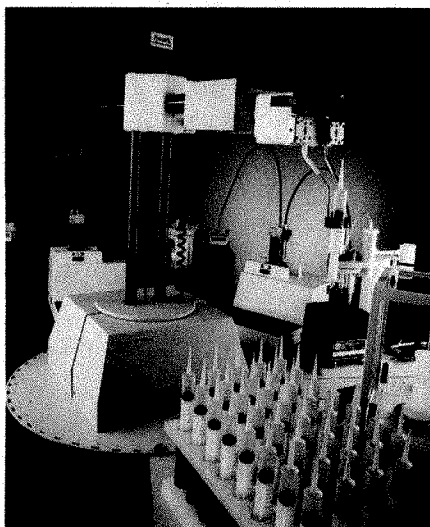
The Pittsburgh Conference will live up to its reputation as one of the largest scientific exhibitions when the doors open in Atlanta, Georgia next week on new products from over 800 companies.

ZYMARK Corporation offers specially configured laboratory robotic systems for automated titrations (Reader Service No. 101). Using Zymark's PyTechnology hardware, the robotic systems can handle the preparation of liquids, viscous liquids and solid samples for Karl Fischer and general titrations. The samples are prepared by the robot, in a sequence that is specified by the operator, and placed directly into the titration cell. The robot starts the titration and monitors all operations to ensure a proper titration. When the titration is done, the results are sent



Zymark's new System V robot controller, for data management and report writing.

back to the robot and a report is generated. Zymark says the robot yields more accurate and precise data than that collected from manual experiments, because its operations are truly reproducible. The company's new System V robot controller performs data management and report writing functions, and archives and transmits data to central computers.



Karl Fischer and general titrations can now be done automatically with Zymark's robot.

from *Schisandra* to be schisanhenol (54 per cent), pregomisin (21 per cent), meso-dihydroguaiaretic acid (13 per cent) and schisanhenol acetate (10 per cent). A total of six lignins were identified: the remaining molecules were nonpolar components with high partition efficiencies in the stationary phase that were not eluted at the same time under the mobile phase conditions.

The coupling of CCC to MS is in its early stages, and new refinements are continually being made. Thick-walled tubing and connections that withstand the high backpressure (roughly 600 p.s.i.) of thermospray MS will make CCC/MS more convenient and easy to use. An analytical CCC system capable of operating at 4,000 r.p.m is being developed in the Laboratory of Technical Development at the US National Institutes of Health which should raise the separation capabilities of the technique. The feasibility of using two aqueous non-immiscible

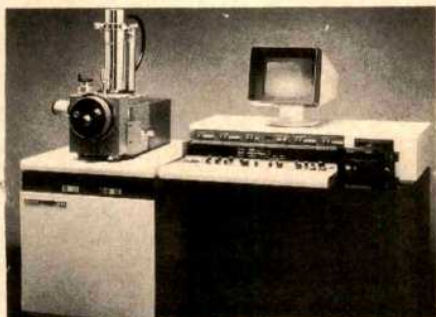
International Scientific Instruments is announcing its new computerized scanning electron microscope — the model SX-40A (Reader Service No. 102). The microscope's evacuation system, high-voltage turn-on function and emission settings are all automated for one-touch operation in sequence. The SX-40A's special features include a motor-driven specimen stage, computer-aided auto-focus and auto-stigmator, and automatic contrast/brightness control. The system operates a

solvent systems for separation of proteins is also being investigated. □

Yue-Wei Lee and Robert D. Voyksner are at the Chemistry and Life Sciences Division, and the Analytical and Chemical Sciences Division, Research Triangle Institute, Research Triangle Park, North Carolina 27709, USA. For more information, fill in reader service number 100.

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ISI's computerized SEM is designed for operation at the touch of a single button.

accelerating voltages from 1 kV to 30 kV, allowing it to be used in both beam-sensitive applications and at high beam energies with an ultimate resolution of six nanometers. Samples of up to six inches in diameter can be examined, with scanning speeds variable from 0.2 to 160 seconds. A full selection of options allows the \$45,000 (US) system to be tailored to specific user requirements and applications.

Sartorius Instruments will introduce its new **PLUS performance package for balances** at the Pittsburgh Conference (Reader Service No. 103). The instrument has a microprocessor-based keypad with a menu of 16 useful functions that expedite common weighing applications. A few of the functions include the storage of identification numbers, calculation of the



Sartorius's PLUS package gives its range of balances a host of special functions.

weight of residue in per cent, a fill-toward-zero capability, and even live animal weighing.

Oriel Scientific Ltd has a new catalogue on **lasers and accessories** which contains a valuable composite of new and existing products, with support to build up applied systems (Reader Service No. 104). Included are both solid state and conventional HeNe lasers, with full details of supporting optics, launching devices, beam splitters, expanders, mirrors, etalons, optical filters, fibre optics and attenuators. Oriel has pitched the free 80-page catalogue to be useful to both research scientists and applied engineers.

### The UV/visible scene

Varian has a new **software interface** program for coupling its Cary 200 series of UV/visible near-IR spectrophotometers

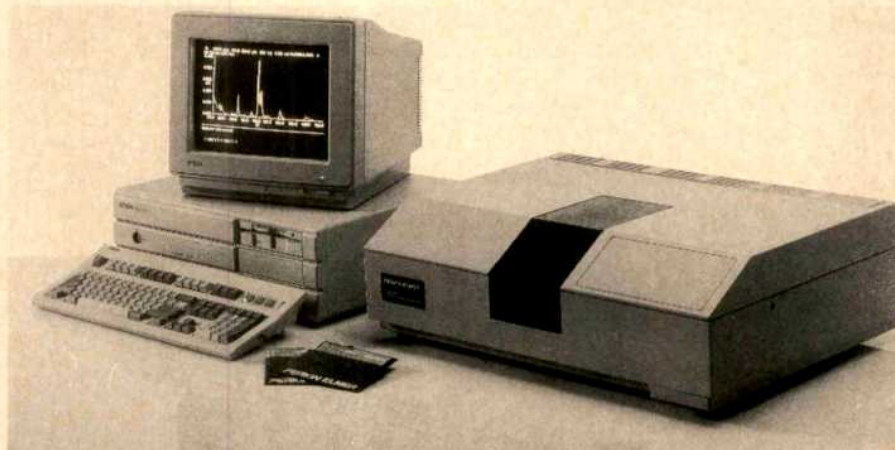
with IBM PC's (Reader Service No. 105). The \$1,250 (US) PC Control program provides centralized control of the spectrophotometer from an IBM PC/AT or PS/2 model 30 or 50. Key features of the package include the ability to store and retrieve instrument parameters, display data in real time, produce multicolor pen plots, and convert data to standard formats usable by other IBM-compatible



A screen display from Varian's software interface for its UV/visible near-IR spectrophotometers.

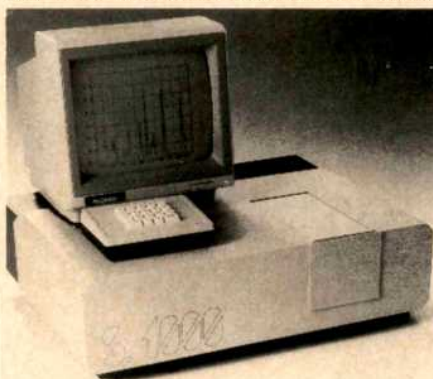
software packages. PC Control has a graphical interface which allows the user to scroll through display options, confirm selections, and move step-by-step through the software.

Perkin-Elmer's **Lambda 6 PC-controlled UV/visible spectrophotometer** was designed for applications in environmental, pharmaceutical and biochemical analyses (Reader Service No. 106). The Lambda 6 PC system is comprised of the double-beam, scanning Lambda UV/visible spectrophotometer integrated with an industrial-standard personal computer and software. Perkin-Elmer says the optical design of the system ensures low stray light, spectral fidelity, fast scan speeds, baseline stability and low noise. The system's software automatically processes and stores data from procedures such as scanning, time drive, wavelength programming, spectral processing and kinetics studies. Its open-ended design allows future expansion to increase data handling capabilities.



Perkin-Elmer's Lambda 6 UV/visible spectrophotometer is controlled by a computer.

Secomam is introducing a **computer-driven spectrophotometer** at the Pittsburgh Conference, designed to scan the entire UV/visible range at speeds of up to 2,400 nm per minute (Reader Service No. 107). In addition to standard measurement modes, Secomam's model S.1000 can perform kinetic measurements at a single wavelength with sampling rates of as high as 30 per second. The system's software can carry out sophisticated calculations — including derivatives up to the fourth order — curve storage, and curve expansion. Up to five components of a single solution can be quantitatively analysed automatically with the system. A multi-standard calibration function permits four different curve fitting models and as many as twelve different combinations of math-



Secomam's S.1000 spectrophotometer also comes with a microprocessor instrument.

ematical axes. The cell compartment can accommodate cells with light paths up to 100mm long, for highly sensitive detection of low level components.

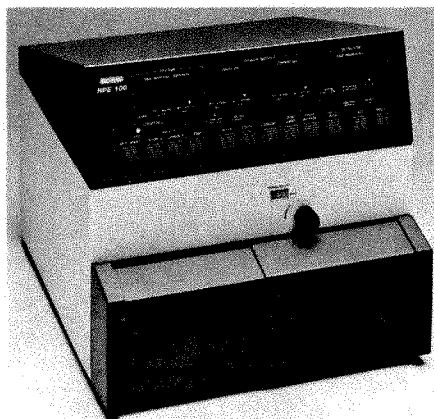
New **quantitative analysis and enzyme kinetics software** is now available from Hewlett-Packard for its model HP 8452A diode array UV/visible spectrophotometer and ChemStation (Reader Service No. 108). The HP 89511A quantitation software package simplifies single component method development and multicomponent analyses. In single component analyses, the software allows the selection of



wavelengths which provide the best calibration curve and the greatest reproducibility. The concentrations of up to twelve components can be determined within a known sample matrix. The software allows the use of absorption and derivative spectra, and the company says it saves time in method development by mathematically synthesizing spectra before measuring actual samples to determine the feasibility of performing the multicomponent analysis. The new enzyme kinetics package, model HP89512A, provides biochemists with the means to investigate enzyme reactions, rate data and reaction models. Nine mathematical models are available, or users can develop models from their own equations. The kinetics of several reactions may be measured in parallel with the help of a new multi-cell transport mechanism for the HP spectrophotometer.

## Capillary electrophoresis

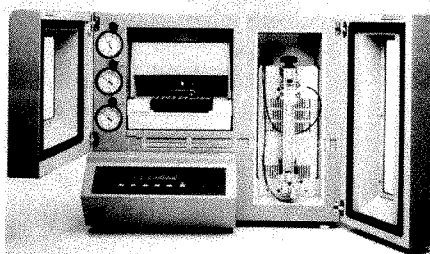
Bio-Rad is now offering an instrument for performing **high performance capillary electrophoresis** (*Reader Service No. 109*). The new model HPE 100 system can perform high-resolution separation and analysis of a variety of samples, including peptides, proteins, DNA fragments, amino acids, bacteria and viruses. The



Bio-Rad's benchtop unit for performing capillary electrophoresis.

system is a compact benchtop unit which contains an electrophoresis chamber and all of the electronics in a safe interlocked cabinet. Separation takes place in a short, narrow-bore capillary tube with a patented coating which Bio-Rad says eliminates electroendosmosis. Samples are injected with a microliter syringe and the electropherogram results are produced on either a strip chart recorder, an integrator, or a PC-driven data collector within about ten minutes, according to the company. Electrophoresis methods that can be adapted to HPE include free zone, displacement and discontinuous methods, and isoelectric focusing.

Applied Biosystems is also introducing a new system for **high-pressure capillary electrophoresis** — its new model 230A



Applied Biosystems's entry onto the capillary electrophoresis market.

HPEC system (*Reader Service No. 110*). The capillary electrophoresis process enables the user to separate, visualize, quantitate and recover mixtures of biological macromolecules. The 230A HPEC is a micro-preparative instrument for the separation and purification of proteins, DNA, and synthetic oligonucleotides. Applied Biosystems says the system offers the resolving power of gel electrophoresis with the convenience of continuous sample elution, real-time visualization and direct sample collection. In addition, HPEC collects peaks of interest for further analysis or purification. As samples are eluted from the tube gel, a continuous flow of elution buffer carries the separated sample components through an on-line detector and into an integral fraction collector. The temperature-controlled gel compartment accepts a variety of column diameters and lengths, and the programmable power supply offers multiple operation modes. The company says standard electrophoresis protocols may be adapted readily to the tube gel format of the system.

## Chromatography currents

Sanki Laboratories has a new model LLB benchtop, high-speed **analytical centrifugal partition chromatograph** (*Reader Service No. 111*). Centrifugal partition chromatography (CPC) is a new liquid chromatographic technique which utilizes liquid-liquid partition and countercurrent distribution to fractionate complex mixtures of chemical substances. Because CPC is not based on a solid stationary support, there is no pH limitation, irreversible retention or catalytic molecular



Sanki's newest analytical centrifugal partition chromatograph runs at 2,000 r.p.m.

rearrangement of labile species. The model LLB's main centrifugal module can be operated at speeds of up to 2,000 r.p.m. The unit contains 8,000 partition channels for the high-efficiency analysis of mixtures ranging from natural products and pharmaceuticals, to antibodies and genetically engineered products.

Dionex has announced what may be the first system for **environmental analysis** to bring together the power of HPLC, ion chromatography and flow injection analysis (*Reader Service No. 112*). The basic system consists of a quaternary gradient module, a chromatography module and a postcolumn module. A column-switching valve allows a single sample to be analysed using various combinations of variable wavelength detectors, fluorescence detectors, a conductivity detector and a pulsed amperometric detector. Post-column derivatization adds the ability to determine contaminant amounts down to p.p.b. levels. The system can be quickly adapted for flow injection analysis by replacing the chromatographic column with a method-specific AppliCard. The fluid path in all of the system components is metal-free, so all types of solvents can be used without corrosion concerns.

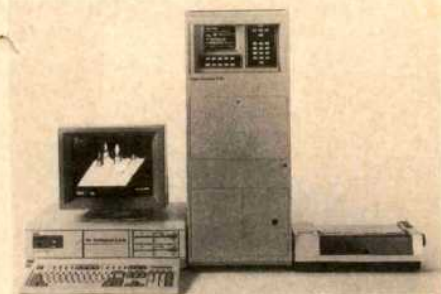
A new **capillary SFC method development kit** is now available from Lee Scientific (*Reader Service No. 113*). The kit contains four 2.5-m, 50  $\mu$ m i.d. columns containing one of each of the following four phases: SB-Octyl-50, SB-Biphenyl-30, SB-Cyanopropyl-50 and SB-Smectic-50. Lee says the 2.5-m columns produce quick results during screening runs, yet deliver efficiencies high enough for the resolution of complex samples, so that they may be rapidly screened to determine which stationary phase is best suited for a particular application.

Perkin-Elmer has a new **liquid chromatography system** which offers computer control for methods development and automated sample processing (*Reader Service No. 114*). The Perkin-Elmer LC Analyst system includes a quaternary LC pump, random-access autosampler, and a personal computer with LC Analyst software. It will accept data from any detector, and is designed to assist chromatographers in the development of methods even when little or no sample information is available. Perkin-Elmer says the LC Analyst's ability to process large numbers of samples automatically and thorough documentation allow developed methods to be transferred easily to routine use.

Special features of the LC Analyst system include customized methods development, software packages, history file reports for the documentation of methods and real-time changes, and automatic systems startup and shutdown.



The Waters chromatography division of Millipore has designed a Peptide Analyzer for high resolution **peptide mapping** (*Reader Service No. 115*). The HPLC system features dual flow paths optimized

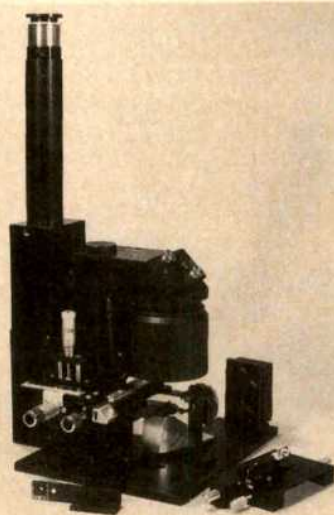


Waters' system for use in peptide mapping.

for microbore and analytical scale operation. The analyzer comes with the Power-Line HPLC controller, which allows single-point control of gradient, injection and detection parameters. The system is also equipped with the Waters model 990+ Photodiode Array Detector which offers complete UV/visible spectral analysis. Spectra can be directly compared to those acquired previously using the detector spectral library. Waters says the system is ideal for the preparation of picomole amounts of pure peptides for sequence determination, peptide maps for the quality control of recombinant DNA products, or for qualitative structural analysis.

### Analytical spectroscopy

Spectra Tech has redesigned its FT-IR Spectra-Scope to allow the **microsample analysis** of crystals, fibres, laminates, con-



Spectra Tech's 'scope has been redesigned for microsample analysis.

taminants, and other small samples difficult to load onto a standard beam condenser (*Reader Service No. 116*). The scope has a 15 $\times$  collecting objective and a 10 $\times$  ocular lens which together magnify the sample area up to 150 times. The company says the Spectra-Scope has a low signal-to-

noise ratio, even with a sample as small as 15  $\mu$ m. Samples are positioned using the X-Y-Z translation stage and light straying is minimized with a remote aperture which masks the image of the sample. Illumination is provided by a high intensity lamp with intensity adjustment. The new \$8,500 (US) Spectra-Scope is designed for use with most FT-IR spectrometers.

Applied Research Laboratories is launching a new version of its thermally stabilized SpectraSpan VII **multi-element spectrometer**, available in either simultaneous or sequential configuration (*Reader Service No. 117*). In simultaneous mode, ARL says the SpectraSpan VII is capable of determining as many as 20 elements in 30 seconds; sequentially, it can determine more than 70. The computerized, bench-top spectrometer is controlled by an IBM PS/2 computer and MS-DOS software that provides operator feedback, generates internal reports, and documents all analytical diagnostic information. The spectrometer features automatic dynamic background correction and high resolution, and a new digital LCD display for gas flow control and profiling functions. The SpectraSpan VII can be used in environmental, clinical, agricultural and geological laboratories.

Philips is introducing its model PW1480 **sequential X-ray spectrometer** — a versatile system for applications requiring high performance and flexibility in the analysis of solids, powders and liquids (*Reader Service No. 118*). Developed from the PW1400 series, a new model incorporates a number of proven features: a high precision goniometer, a 60 kV or 100 kV generator, and a wide range of single- and dual-anode X-ray tubes. With the model PW1480, Philips has added a wider choice of collimator and crystal options, plus a bidirectional, eight-position crystal changer to facilitate custom tailoring. To go with the spectrometer, Philips offers its new X-40

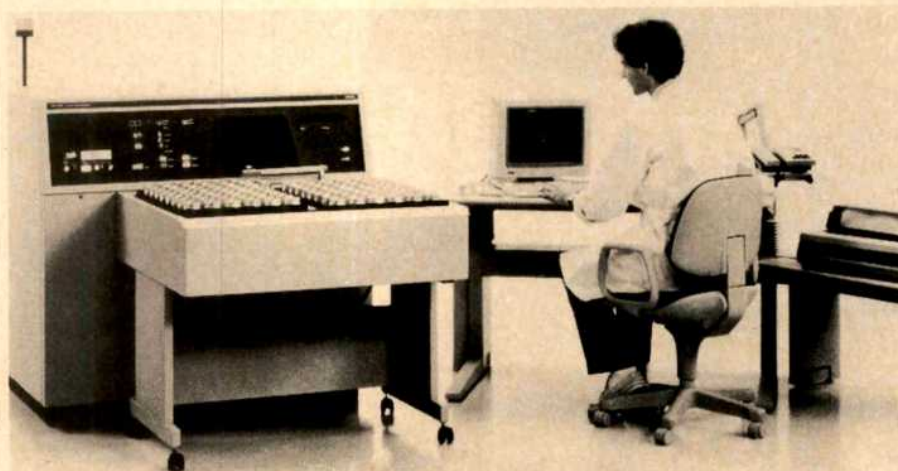
analytical software package, which has facilities for both quantitative and qualitative spectrometer operation, and a user interface which supports both the command and menu modes. To make the software easier to use, Philips has included logical defaults at input points throughout the program and easily accessible "help" information. Philips says the software package reduces calculation time by allowing automatic qualitative and semiquantitative analysis of several samples with a single command.

Jeol has a new **mass spectrometer with ion optics** which it says is as equally suited for high-sensitivity, high-resolution GC/MS as it is for high-molecular weight



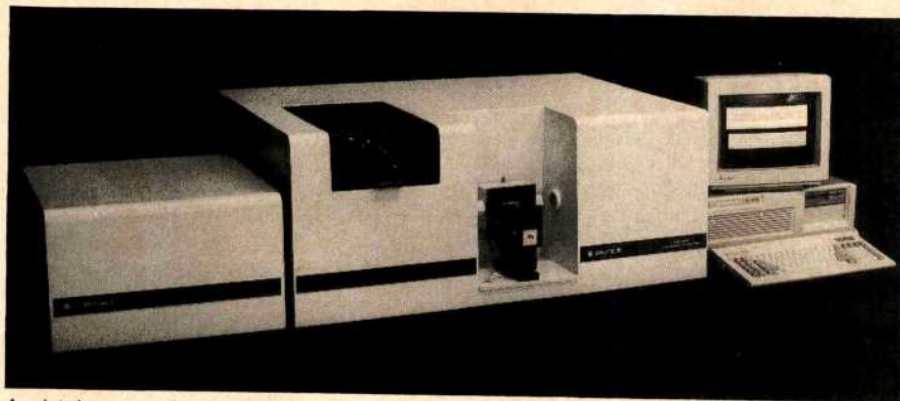
Ion optics of Japanese design enhance Jeol's new mass spectrometer, the model SX-102.

LC/MS measurements (*Reader Service No. 119*). The Jeol SX-102 can be used to detect very small traces of drugs, pesticides and pollutants with the SIM GC/MS technique. Its LC/MS applications using the frit inlet system include the analysis of amino acids, peptides, proteins and pharmaceuticals. The instrument's QHQ optics geometry, created by Matsuda of the University of Osaka in Japan, provides high ion transmission and positively shapes the beam to allow a slit width five times that of conventional mass spectrometers, for high sensitivities at both low and high resolution. The instrument has a practical mass range of 3,000 daltons at 8 kV, but Jeol says it can measure compounds of greater than 5,000 daltons, such as bovine insulin.



Philips' sequential X-ray spectrometer can handle solids, powders and liquids.



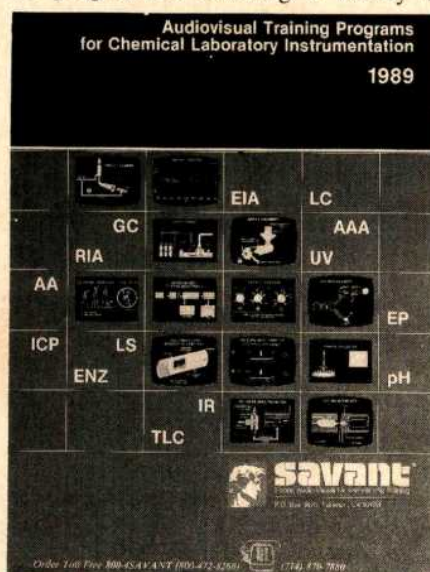


Analyte's answer for multielement atomic absorption spectrophotometry.

Analyte Corporation is introducing its new model SL-16 **multielement atomic absorption spectrophotometer**, which the company says is capable of determining in rapid sequence the concentrations of up to 20 elements in either liquid or solid samples (*Reader Service No. 120*). The SL-16 contains a fast-moving turret which holds 16 hollow cathode lamps combined with a monochromator programmed to move to the correct wavelength while the turret is rotating. Analyte claims the system can determine 20 elements from one sample in under two minutes. Conventional burner systems can be used with the spectrophotometer for liquid samples; Analyte's Atomsource glow-discharge device is required to atomize solid samples into the argon gas stream. The Atomsource can be connected to the spectrophotometer in roughly 10 minutes. The SL-16 is controlled by an AT-type computer that can be programmed with up to 400 different analytical methods, each of which includes lists of elements, wavelengths, and slit widths.

### Information resources

Savant's 1989 catalogue of audiovisuals for **chemical laboratory training** contains 76 programmes covering a variety of



Savant's catalogue on tutorials.

analytical techniques for both industrial and medical applications (*Reader Service No. 121*). Available training programmes range from peptide analysis and UV/visible spectroscopy, to general laboratory procedures and chemical safety. Most Savant programmes are available as both slide and cassette tape shows and videotapes in Beta or VHS formats. The programmes are professionally narrated, and vary in length from 20 to 45 minutes.

The Sigma-Aldrich Company has published the second edition of its two-volume library on **chemical safety** (*Reader Service*



Sigma-Aldrich's volumes contain things chemists need to know for a safer lab.

*No. 122*). Over 14,000 chemicals and 24,000 products are listed in the two books, with information ranging from chemical structure and physical properties to health hazards and recommended waste disposal methods. The volumes together contain 4,098 pages, and cost \$325 (US).

Finnigan MAT is offering a free **colour wallchart** to be used in the interpretation of peptide and protein mass spectra (*Reader Service No. 123*). The information provided on the wallchart includes amino acid structures, molecular weights, and hydrophobicity indexes. The wallchart also lists the gas phase fragmentation pathways of peptide chains and the differences between their nominal, average and exact molecular weights. The colour wallchart is the first of a series

planned by Finnigan MAT to explain the biochemical applications of mass spectrometry; the company will introduce additional wallcharts later this year.

To complement its "How To" notes detailing applications and techniques for using FTIR spectrometers, Mattson



Mattson's FT-IR spectroscopy videotape.

Instruments Ltd is releasing an **education film** entitled "Infra-Red Spectroscopy" (*Reader Service No. 124*). The video-cassette film is produced in conjunction with the British Broadcasting Company's Open University programme, and offers both the established and the potential user theoretical and practical information on the FTIR spectroscopy technique. A range of methods for the measurement of gas, solid and liquid samples are demonstrated on the video, using Mattson FTIR spectrometers.

The 1989 **catalogue** is now available on the Eyela line of scientific instruments from Tokyo Rikakikai Company Ltd. (*Reader Service No. 125*). The 45-page catalogue lists a host of products for analytical chemistry, cell culture, microbiology, and biotechnology. Besides such basic laboratory items as freeze-dryers, water baths, incubators, ovens and water purifiers, the Eyela catalogue spells out Tokyo Rikakikai's specialty systems for laboratory- or production-scale rotary evaporation, cell immobilization and carboxylic acid analysis. The catalogue also describes the company's line of batch and airlift bioreactors for plant and animal cells, and fermentation pilot plants. For chromatography needs, the catalogue includes information on the Eyela droplet counter-current chromatograph, flash chromatograph, rotation locular counter-current chromatograph and liquid-liquid counter-current extractor.

Beckman is introducing its new **Data Logger software for spectrophotometry** at the Pittsburgh Conference, and is exhibiting the package along with one of its DU Series 60 spectrophotometers (*Reader Service No. 126*). Data Logger allows users to log discrete absorbance readings of up to 114 samples on 3.5-inch diskettes, collect wavelength scans, carry data to





Beckman's new Data Logger software expands the capabilities of its spectrophotometers.

another IBM PC or PS/2 personal computer for analysis, and convert data to Lotus format. With the software, users can add or subtract spectra, and display up to six spectra on the screen for direct viewing and comparison. The Data Logger package includes an IBM PS/2 model 25 computer with 640 kBytes RAM and dual 3.5-inch disk drives, DOS version 3.3, the Beckman DU Data Capture software program, a formatted data diskette and necessary cables. Data Logger also supports the Beckman DU-50 and DU-70 series spectrophotometers, and the DU-6 and DU-7 UV-visible spectrophotometers.

### Biology at Pittcon

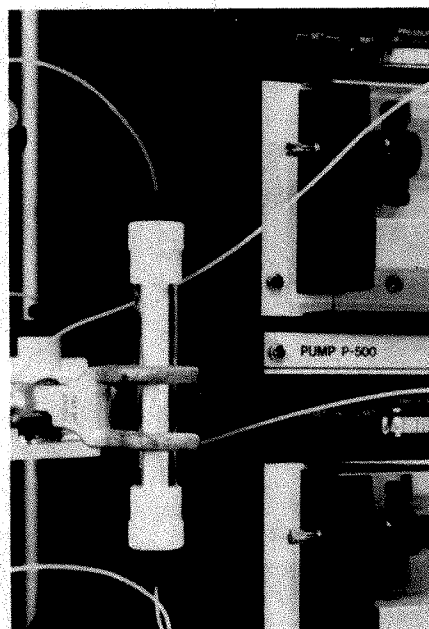
OROS Systems, Inc. is launching its second instrument for **protein purification** — the touch-screen operated MultiLab system (*Reader Service No. 127*). OROS designed the MultiLab for use in small-scale preparative purifications and for determining optimal purification methods. The operator chooses the preferred method by touching the MultiLab's touch-sensitive screen, and the expert software system takes over to monitor the purification and control all parameters. The \$35,000 (US) MultiLab runs at both low and medium pressures, and includes air sensors to detect the absence of buffers and an air eliminator to protect the column. OROS Systems says the system can be used with immunopurification, affinity chromatography, hydrophobic interaction chromatography, ion-exchange, gel-filtration or chromatofocusing techniques.

The Generator **DNA synthesizer** will be on display in DuPont's booth at the Pittcon show (*Reader Service No. 128*). The \$22,500 (US) Generator uses  $\beta$ -cyanoethylisopropyl phosphoramidites, which DuPont says demonstrate consistently high base-to-base coupling efficiencies. The precision fluid meter of the Generator delivers microlitre quantities of reagent to the reaction column to save reagents, and can perform at least 100 coupling cycles before requiring replenishment, according to the company. The Generator has a built-in system check which alerts the user and places itself on hold if either fluid flow or gas pressure fall outside set parameters. Operating the system requires no computer language

skills: nucleotide sequences are entered directly from the keyboard, and the screen display shows the status of the reaction cycle and the base position in the sequence. A custom programming function allows users to develop their own chemistry programs, which can be stored on disk through the system's floppy disk drive. DuPont offers a complete range of pre-measured reagents for use with the Generator which come in thumb-screw-on bottles that cannot be misinstalled.

Polymer Laboratories Ltd have a new line of **LC columns** for protein analyses which link the company's biocompatible metal-free column with a range of quaternized PL-SAX strong anion exchange materials (*Reader Service No. 129*). The PL-SAX media are composed of a quaternary amine applied to a rigid macroporous polymer, giving a pH range of 1–13 and a high degree of physical stability.

Molecular Devices recently introduced the first laboratory instrument to employ biosensor technology — the **Threshold total DNA assay** system based on the light-addressable potentiometric silicon sensor developed by Hafeman, Parce and McConnell (*Reader Service No. 130*). The Threshold system quantitates contaminating DNA in samples of biotechnology products, down to the picogram level. In the first step, the sample is incubated for one hour with a single reagent that contains two binding protein conjugates: a single-stranded binding protein linked to a hapten specific for the capture membrane, and an anti-DNA monoclonal antibody joined to urease. The sample is then transferred to the vacuum unit of the Threshold, which concentrates it onto eight measurement sites on the Threshold biosensor



Polymer Lab's all-glass setup for the chromatography of biomolecules.

stick. When the stick is dipped into the reader, it comes in contact with urea, and the ensuing enzyme reaction changes the local pH at each measurement site, which alters the surface potential of the sensor proportionally to the amount of DNA present. Molecular Devices says all DNA fragments larger than 100 bases can be quantitated with the computer-driven Threshold system, which can process up to 32 samples in roughly 15 minutes. The company is currently developing other DNA probe and immunoassay kits for use with the Threshold.

### Japanese Imports

Asahi Chemical sells a **desk-top desalinator** called the Micro Acilyzer based on electrodialysis with an ion-exchange membrane (*Reader Service No. 131*). The electrodialysis technique employs a pair of cation- and anion-exchange membranes which are activated by an electric potential to transport negatively and positively charged ions out of the sample. Asahi says its Micro Acilyzer can be used for the desalination of oligosaccharides, and the removal of phosphate buffer from peptides and nucleosides. The company sells two versions of the Micro Acilyzer: model G1 processes 10 ml samples, and model G3 can desalinate up to 1,000 ml.

The model SDM5500 **dynamic mechanical spectrometer** from Seiko Instruments can be used for the measurement of the physical properties of solid materials (*Reader Service No. 132*). The instrument consists of a rheometer, an analysis module and an X-Y plotter. Stress from the force generator is applied by a probe, and the strain caused in the sample is measured as the displacement signal by a strain meter, and sent to the computer for processing. The system's software incorporates windows so that running conditions and data can be viewed simultaneously. □

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**LIPOSOMES**

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Our new LIPOSOMAT guarantees rapid (5ml within 60 minutes) preparation of uniformly sized unilamellar liposomes. Liposome size is selectable between 25 and ca. 600 nm diameter. Sample volume is normally 5–10ml or up to 200ml if used in combination with an additional instrument. Lipid concentration can be up to 300 mg/ml.

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Reader Service No.45



# Move the work or move the people?

Richard Pearson

Pressures on the crowded and expensive south-east corner of England are leading to 'satellite offices' and long-distance commuting. Many companies see relocation as the only answer.

DESPITE a fast-growing economy, great regional disparities remain in the United Kingdom. In many of the inner cities and towns in the North, unemployment remains stubbornly high, often reaching levels in excess of 15 per cent. Yet in many parts of the South-East, registered unemployment has fallen to under 5 per cent and skill shortages are growing worse. In an effort to alleviate the worst effects of these shortages employers are now looking to recruit from farther afield, in some cases extending their catchment areas by a few miles, in others encouraging long-distance commuting from several hundred miles away. Others are setting up remote networking centres while others are relocating in order to find the staff they need.

A recent study of the South-East has shown the different stages of distance recruitment clearly<sup>1</sup>. The sizes of the individual local catchment areas vary greatly, increasing with the seniority of the appointment, the nearness to the centre of London, and to a lesser extent transport links. The better the links the greater the distances people are prepared to travel.

## Perks?

The first response of employers to shortages is to consider applications from beyond the usual spatial area. This usually involves advertising in the local media, and is a fast, easy, low-cost option. Companies are often reluctant to make even this simple move, fearing poor time keeping and increased absenteeism as a result of long-distance travel. In London, 64 per cent of the professional staff surveyed spend more than an hour travelling to work, compared to 7 per cent in the rest of the South-East and even fewer in some other parts of the country (see table). Towns such as Bristol, Coventry, Doncaster and Ipswich, are now joining the South-Coast resorts such as Brighton and Eastbourne as regular commuter towns for London. Surprisingly, outside of the construction industry, few firms offer improved financial or other assistance with commuting costs such as season ticket loans or car purchase schemes. These are still seen as perks rather than as recruitment aids.

The next step for recruiters is rather bigger and involves advertising in more distant labour markets. Here, a major problem is choosing which labour market might be appropriate, the main indicators used being the prevailing level of local unemployment, local employment struc-

ture and reports of redundancies in firms similar to their own. Firms with branch networks, however, are able to use them as information sources and recruitment agents, and also encourage staff transfers into the South-East. High-tech firms tend to use consultants.

## House prices

There remains, however, a growing reluctance on the part of individuals to move into London and the South-East, with regional house price differentials being a major barrier<sup>2</sup>. In contrast with their reluctance to improve the assistance for longer distance commuting, most firms surveyed are improving their relocation package, although only a minority are investing in sheltered housing, and assisting in property disposal and search, property leasing schemes and joint equity schemes. The numbers of staff benefiting from the latter are small. For most companies the response is financial, providing reimbursement for the costs involved in the move as well as for higher costs in the new area, and supplements to compensate for the disruption. Few companies have detailed knowledge of the real costs involved, but their estimates of expenditure were in the range £5,000-£10,000 with a tax exemption

opportunities. About half of them were employed on temporary or short-term contracts, few were allowed to adjust their working patterns to fit in with their travel arrangements. The extra costs of this travelling tend to fall on the individual, with little subsidy or inducement coming from the employers<sup>3</sup>.

Another variation on long-distance working that is now being pioneered in the United Kingdom is that of network information technology centres in inner cities, remotely serving customers in the South-East. Recruitment to these centres is likely to be from local training schemes and it is intended that the centres will not only be commercially viable, but will also provide valuable work experience and further training to those who would be otherwise employed, and boost the skills profile of the local community. The technology is already proven, the key is to attract the customers. In the United States a number of companies now send all their low-level data processing work via satellites to centres in the Caribbean where wage rates are way below those of the United States — in some cases below even the cost of office rental in North America.

An increasing number of employers, including central government, are also

Travel-to-work times in selected regions

Journey time	London	South-East	North	Wales
0-29 min	7%	62%	57%	72%
30-59 min	29%	30%	36%	26%
60+ min	64%	7%	7%	2%

Data from ref. 1

limit of £8,000 last year. (Tax exemption is now being increased to £17,200.)

Although many of the available jobs are in the South-East, financial, housing and social reasons often mitigate against a move to the region. Now there is a growing class of professional long-distance commuters, with as many as 10,000 people regularly travelling from the North to work in London and the South-East. Interviews with a small sample of these commuters show that many are middle managers continuing in jobs or careers that they had had earlier in their lives, but that they are now earning far higher salaries than they were previously and demand high levels of job satisfaction. A major motivation for many 'long-distance commuters' interviewed was a history of redundancy, not that of long-term unemployment, and the lack of local job

moving the whole workplace and setting up offices outside the South-East to capitalize on their property assets, at the same time alleviating skill shortages. With labour supply and working conditions unlikely to improve in the South East in the foreseeable future, relocation of the work, whether in terms of the whole office or just part of the workload via telecoms links, looks like a more effective long-term solution than trying to attract more people to travel or move to the work. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton BN1 9RF, UK.

1. *Relocation and Recruitment Difficulties of Employers in the South East* (Institute of Manpower Studies, 1988).
2. *Nature* **332**, 98 (1988).
3. *Britain's New Industrial Gypsies* (Policy Studies Institute, 1989).

**LONDON:** Julie Skeet, 4 Little Essex Street, WC2R 3LF Telephone 01-836 6633 (Telex 262024)  
**NEW YORK:** Marianne S. Ettisch, 65 Bleecker Street, New York, NY 10012 — Telephone (212) 477 9625  
**SAN FRANCISCO:** Megan Van Peebles, Suite 1408, 582 Market Street, San Francisco, CA 94104 (415) 781-3803, 3804 or 3801  
**TORONTO:** Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423  
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### THE UNIVERSITY OF CHICAGO

#### Department of Biochemistry & Molecular Biology **POSTDOCTORAL FELLOW POSITION, STRUCTURE AND FUNCTION OF RAS PROTEIN**

A position is available to study biochemistry of ras proteins. (1) C-terminal modification and membrane association of ras and other CAAX box containing proteins. The study will utilize yeast system and will focus on palmitoylation, carboxymethylation, proteolytic cleavage and farnesylation. (2) Guanine nucleotide binding and GTPase activation of ras proteins and their interaction with GAP.

Send curriculum vitae or call 312-702-1339, **Dr. Fuyu Tamanoi, Department of Biochemistry and Molecular Biology, The University of Chicago, 920 E. 58th St., Chicago, IL. 60637.** (NW3389)A

### Postdoctoral positions

available immediately for Biochemists, Biophysicists and Molecular Biologists to study (1) The Mechanism and Regulation of Gene Expression in both Prokaryotic and Eukaryotic Systems, and (2) the Nucleic Acid-Protein Interactions. Send resume and three letters of recommendation to **Professor Cheng-Wen Wu, Dept. of Pharmacological Sciences, SUNY Stony Brook, Stony Brook, NY 11794-8651.** SUNY Stony Brook is an affirmative action equal opportunity educator and employer. AK 32. (NW3380)A

### UNIVERSITY OF BRISTOL

Faculty of Engineering

#### **Lectureships in Computer Science**

Two posts are available: one appointment will be made in the area of logic programming and artificial intelligence (ref. JPB25) and the other in the general area of parallel computation (ref. JPB26).

Parallel computation, which includes the design and implementation of parallel computing systems and also their applications, is seen as an area of major importance and, within the Faculty, there are groups addressing both fundamental design problems, especially for logic programming systems, and also specific application areas, in particular computer vision. The successful candidate will be expected to join one of these groups.

For the post in logic programming, applications are invited from candidates whose research interests would strengthen existing research activity in logic programming, which includes language design, knowledge base systems, theory of logic programming and natural language processing.

Starting salary in the range £9,260-£14,500 per annum.

For further details telephone Bristol 303136 (ansaphone after 5pm) or write to the **Personnel Office, Senate House, Bristol BS8 1TH.** Please quote the reference of the post which interests you.

Closing date 20 March 1989.

(8565)A

## **Asthma Research in Sandoz, Basel/Switzerland**

The asthma research programme at Sandoz seeks to relate clinical pharmacology and clinical experience of anti-asthma drugs to pharmacology of laboratory mammals when subjected to acute provocation stimuli (allergen, cytokines, PAF or pharmaceuticals) or to chronic exposure (asthmagenic chemicals, PAF, allergens or infectious agents). Measurements of pulmonary function, eosinophil accumulation, mucociliary clearance and novel tests involving platelet activation are used for drug evaluation. Considerable emphasis has been given to electronic capture and processing of data, so that work stations are integrated as a local network and will be linked to complementary laboratories within Switzerland, USA and Japan. A primate laboratory equipped to clinical intensive care standards has been established in order to relate preclinical research to clinical pharmacology and a long-term collaborative relationship has been initiated with a clinical research group specialising in asthma, based in the Swiss Institute of Allergy and Asthma Research in Davos.

Expansion of the asthma research programme in Sandoz necessitates appointment of three post-doctoral scientists to join the preclinical asthma research group.

Applicants (aged less than 35) will be expected to direct research projects and hence should have undertaken a period of independent post-doctoral research.

**PHARMACOLOGIST** to investigate airway hyperreactivity. A background in pulmonary or platelet pharmacology or physiology would be appropriate.

**PHARMACOLOGIST** to investigate the mechanism of eosinophil accumulation and activation with particular emphasis upon the interaction between interleukins and PAF in such processes.

**BIOCHEMIST** to establish molecular mechanisms that could explain efficacy of novel anti-asthma compounds with both prophylactic and bronchodilator efficacy. Close collaboration with chemists and pharmacologists and a willingness to consider a wide range of biochemical options will be necessary to fulfill the promise of this new post

Applications including curriculum vitae should be sent to:

**Sandoz Ltd., Personnel Department, ref 9801,  
 attention Mr R. Zbinden,  
 CH-4002 Basle, Switzerland**



(W5913)A

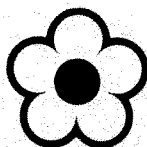


## Royal Postgraduate Medical School

(University of London)

### MRC/LRF LEUKAEMIA UNIT

## MRC Scientist Molecular Basis of Leukaemia



Applications are invited from suitably qualified candidates to lead a molecular biology group within the Unit. Applicants should have a strong background in any area of molecular or cell biology and a desire to understand leukaemogenesis and the pathophysiology of leukaemia cells.

The MRC/LRF Leukaemia Unit has recently been expanded and its scope developed to include investigations into the molecular basis of leukaemia.

The Unit includes the LRF Centre for Adult Leukaemia and is closely integrated with the clinical service and the laboratory of molecular genetics in the Department of Haematology, within which the Unit is located.

The successful candidate will be offered a fixed-term or career appointment according to qualification and experience, with remuneration at an appropriate point for University non-clinical scientific staff.

Postdoctoral positions may also be available.

Informal enquiries may be made to the Unit Director, Professor L Luzzatto, on 01-740 3234.

Candidates are invited to apply with a curriculum vitae, names of three referees and a statement of research plans to the **Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0NN (01-740 3204)**, quoting ref: MB/LRF.

Closing date: (1 month).

(8559)A

## UNIVERSITY OF BRISTOL Lectureship in Physics

Applications are invited for the post of Lecturer in Physics. It is hoped to appoint an experimentalist with interest and experience in the physics of liquids and amorphous solids.

The appointment will be on the salary scale for Lecturers, currently £9,260–£19,310 per annum.

Applications should be made by letter stating special academic and research interests and should include the names and addresses of three referees, and should be accompanied by a full curriculum vitae. Applications should be sent, quoting reference JPB/36, by 23 March 1989 to the **Registrar, University of Bristol, Senate House, Bristol BS8 1TH** from whom further particulars about the post and the research work at present in progress may be obtained.

(8552)A

## ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

### DEPARTMENT OF CHEMICAL PATHOLOGY A Grade 1A Post-Doctoral Scientist

is required for a 3 year MRC-funded project grant to write a novel cell-surface molecules involved in the adhesion of T lymphocytes to various targets. Experience in monoclonal antibody technology and T cell clone biology would be valuable.

Salary range £13000–£16000 (inclusive of London weighting).

For further information contact: Dr M W Makgoba  
01-743 2030 ext. 2118.

Proposed starting date: mid April 1989.

Application forms and further particulars available from the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN** (tel: 01-740 3204) quoting ref: AC/MRC.

Closing Date: 16th March 1989.

(8589)A

## SCOTTISH CROP RESEARCH INSTITUTE Invergowrie, Dundee DD2 5DA RE-ADVERTISEMENT

### HIGHER SCIENTIFIC OFFICER (BAND I) POTATO AND BRASSICA GENETICS DEPARTMENT

A vacancy exists for an HSO within the Potato and Brassica Genetics Department of SCRI. The Department is responsible for the largest state supported potato genetic research programme in the UK.

The appointee will join a multidisciplinary team working in all aspects of the genetics of the potato. He or she will be expected to initiate research into the use of true (botanic) seed of the potato as part of a new strategic initiative in this area and collaborate with colleagues in other ongoing programmes.

Salary: £9,219–£12,505

Non-contributory superannuation.

Qualifications: First or upper second class honours degree with at least two years post-qualifying relevant research experience which may be a course of research and study leading to a Ph.D. degree.

The Institute is an equal opportunities employer.

Curriculum vitae complete with the names and addresses of three referees should be sent to **Mr I Paxton, Personnel Officer**, by 17th March, 1989, quoting the appropriate reference PBG/88/4/N (8574)A

## ROOSEVELT UNIVERSITY DEPARTMENT OF BIOLOGY FACULTY POSITIONS IN GENETICS AND VERTEBRATE BIOLOGY

The Department of Biology, Roosevelt University invites applications for two assistant professor, tenure-track positions. Exceptionally well-qualified candidates may be considered for appointment at the associate professor level. Applicants for the genetics position should be able to offer an introductory genetics course and advanced undergraduate and graduate course work in their speciality. The ability to offer a graduate biostatistics course would be valuable. Applicants for the vertebrate biology position should be able to offer a vertebrate biology course and advanced undergraduate and graduate courses in their speciality. The ability to offer an undergraduate developmental biology course would be valuable. Both positions also entail offering portions of the departmental core courses.

Roosevelt University is a small, privately-funded, urban university located in downtown Chicago and at two suburban locations. Applicants should have a Ph.D. and a strong interest in undergraduate and master's level teaching as well as a commitment to developing an independent research program.

Please send your curriculum vitae, a summary of teaching abilities and a description of your research interests and arrange to have three (3) letters of recommendation submitted by April 1, 1989 to: **Dr. Jonathan Green, Chair, Department of Biology, Roosevelt University, 430 South Michigan Avenue, Chicago, Illinois 60605.**

Affirmative Action/Equal Opportunity Employer

(NW3387)A

## UNIVERSITY OF BRISTOL DEPARTMENT OF PHARMACOLOGY Lectureship in Molecular Pharmacology

A Lectureship in Molecular Pharmacology is available under the NAAS Scheme. Applicants will be expected to assist in a broad range of teaching to science and medical undergraduates and in advanced teaching to final year Honours students. It is hoped that the person appointed will have research interests in an area of molecular pharmacology with experience in molecular graphic techniques being preferred but not essential.

Pursuit of the candidate's research interests will be strongly encouraged and it is hoped that he/she will work in association with the newly established Molecular Recognition Initiative in the Bristol Medical Sciences Building.

The NAAS Scheme is intended to create career opportunities for young academic staff at an early stage in their careers.

Starting salary in the range £9260 – £14500 per annum.

For further details telephone Bristol 303136 (ansaphone after 5pm) or write to the **Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference TLJ3.**

Closing date 20 March 1989.

(8564)A



## **GS-13 Biochemist**

### **Biological Response Modifiers Program, DCT, NCI**

The Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, is accepting applications for a biochemist position.

This position is available for an outstanding Ph.D., M.D. investigator with expertise in protein purification and analysis procedures as well as a strong background in biochemistry and molecular biology. Incumbent will serve as a Senior Investigator responsible for planning biological, immunological and biochemical approaches to the questions of lymphocyte regulation of normal and abnormal human leukocyte development and function. Incumbent will exercise a high degree of independent judgment as to formulating research approaches and supervising other members of his/her laboratory research staff in attaining these objectives. Responsibilities include developing and teaching other members of his laboratory research staff as well as outside collaborators the techniques necessary to study the role of lymphocytes in the growth and maturation of normal leukocytes and their possible therapeutic use in man. Research interests should focus on analysis of the role of several important regulatory molecules, such as interleukin 1, tumor necrosis factor and chemotactic factors in the activation and development of biologically mature, normal leukocytes; the purification, identification and molecular cloning of new factors which affect leukocyte development, maturation and function; the production, and mechanisms by which these proteins are processed into functional molecules; the interactions of these proteins with substrates in the target cells; and the development of monoclonal and polyclonal antibodies to these target proteins for use in more sensitive assays and as screening

reagents for human leukemias. The incumbent must possess extensive experience in and thorough knowledge of all aspects of lymphocyte biology (production, purification, assays, functions) and immunology and demonstrate research potential and accomplishment as evidenced by scientific publications. Experience with application of basic research findings to clinical situations, in order to pursue the work along clinically important lines, are critical for the success of the research.

The position will be a Civil Service position with an annual salary between \$41,121 and \$53,460 (based upon experience). Incumbent will be eligible for all benefits including health insurance, life insurance options, vacation and sick leave.

Applicants should submit CV and three letters of recommendation to:

**Dr. Joost J. Oppenheim**  
**Chief, Laboratory of Molecular**  
**Immunoregulation**  
**Biological Response Modifiers**  
**Program**  
**Division of Cancer Treatment**  
**National Cancer Institute**  
**Frederick Cancer Research Facility**  
**Building 560, Room 21-89A**  
**Frederick, MD 21701-1013**  
**Telephone: (301) 698-1551**



U.S. CITIZENSHIP IS REQUIRED  
NIH IS AN EQUAL OPPORTUNITY EMPLOYER

*Deadline for applications is April 30, 1989.*

## PROGRAM OF EXCELLENCE IN MOLECULAR BIOLOGY

### New Investigator Positions

The National Heart, Lung and Blood Institute of the NIH has awarded the University of Cincinnati Medical Centre a "Program of Excellence in Molecular Biology". The goals of the University of Cincinnati Program are to provide skills development and research opportunities in the molecular biology of heart and lung. Specifically, the Program will concentrate on gene regulation and structure-function analysis of proteins which play a central role in heart and lung. In addition, the role these genes play in human health will be investigated. The skills development program includes a 5 year program, initiating at the postdoctoral level and progressing to junior faculty status in the final years. New Investigators will initially work closely with the sponsor but in the final year will develop a research problem separate from that of the sponsor, and apply for independent funding to enable the Investigator to move to another institution if desired.

We are presently recruiting 12 such individuals with an additional twelve to be added in the second year. Ph.D.s or M.D.s with a strong background in molecular genetics who wish to apply their skills to significant problems of heart and lung or individuals with strong backgrounds in heart and lung who wish to develop molecular genetics tools for continued study of these problems are encouraged to apply. We are particularly interested in recruiting minorities to the Program. Stipends are competitive, beginning at £25,000 per year and moving to \$37,000 in final years. The core faculty are:

1. **Jerry Lingrel, Ph.D.** — Regulation of the Na,K-ATPase genes and RFLP analysis of diseases such as familial hypertension. Identification of the cardiac glycoside binding site using site specific mutagenesis.
2. **Jeffrey Whitsett, M.D.** — Characterization of lung surfactant proteins and genes.
3. **Winston Kao, Ph.D.** — Collagen gene expression in the lung.
4. **Gary Shull, Ph.D.** — Structure-function relationships and genetic regulation of Ca-transporting ATPases of intracellular and plasma membranes.
5. **Jeffrey Robbins, Ph.D.** — Study of cardiac contractile proteins and growth and developmental control of muscle and pulmonary proteins.
6. **Donal Luse, Ph.D.** — Transcriptional analysis of lung surfactant protein gene promoters.
7. **Steven Potter, Ph.D.** — Insertional mutagenesis for identifying critical genes in heart and lung development.
8. **Arnold Schwartz, Ph.D.** — Structure and function of Ca<sup>++</sup> channel proteins and gene regulation; isolation of receptors for calcium antagonists.
9. **Judith Harmony, Ph.D.** — Proteins and genes involved in neutral lipid transfer; the regulation of cell proliferation and differentiation by apolipoproteins.
10. **Thomas Doetschman, Ph.D.** — Gene targeting in ES cells using homologous recombinations. Focus will be on vasculogenesis, angiogenesis and cardiogenesis.

Applicants should submit a C.V. and the names of three references to **Jerry B Lingrel, Ph.D., Director, Program of Excellence in Molecular Biology, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267-0524.**

*Affirmative Action/Equal Opportunity Employer*

(NW3379)A

## CALIFORNIA INSTITUTE OF TECHNOLOGY

### Junior Faculty Position in Geology

Applications are being accepted for a tenure-track Junior Faculty position in Geology in the Division of Geological and Planetary Sciences. The initial appointment is for four years. It is possible that a candidate with exceptional qualifications may be considered for a more senior position. Outstanding individuals with a strong commitment to original research and teaching in any areas of geology are encouraged to apply. We are particularly interested in scientists whose work relates to 1) tectonics/geophysics/structural geology, with special emphasis on the observational attack on current geologic processes as exemplified by those occurring over the past million years; and/or 2) the structure and origin of the deep continent crust. A bridging between geology and geophysics, to achieve the full advantages of applying geophysical understanding in field-oriented geological studies, is a highly desirable feature of the contemplated appointment. Individuals with potential for interactions with existing programs in geophysics and geochemistry are encouraged to apply.

A curriculum vitae including a list of publications and a brief description of proposed research activities should be sent to **Professor G. J. Wasserburg, Chairman, Division of Geological and Planetary Sciences, 170-25 (APA), California Institute of Technology, Pasadena, California 91125.**

*The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer.* Applications are encouraged from qualified women and minority candidates. (NW3376)A

## UNIVERSITY OF EDINBURGH

### HIV RESEARCH

#### DEPARTMENTS OF CHEMISTRY AND GENETICS

### A Postdoctoral position

is available immediately for work on an investigation of the inhibition of HIV by phosphorothioate oligonucleotides, directed by Dr. Tom Brown (Chemistry) and Drs. A. J. Leigh Brown and J. O. Bishop (Genetics). Two main lines of research are planned: (1) An investigation of the mode of action of phosphorothioate and other oligonucleotides and (2) The identification of novel oligonucleotides with greater inhibitory effects in relation to cytotoxicity. Candidates should have a background in cell biology and preference will be given to those with experience of molecular biology and/or animal virology.

The appointment is available for three years, with salary in the range £9,865-£15,720 with maximum starting point of £13,870 for a suitably experienced candidate. Enquiries may be directed to 031-667-1081, Extension No. 3448 (T. Brown), 3551 (A. J. Leigh-Brown) or 3553 (J. O. Bishop), and applications, with full C.V. and names and addresses of two referees, should be sent to **Mr. A. Gillies, Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh EH9 3JN, Scotland.** Please quote reference No. 5641. (8577)A

## UNIVERSITY OF BRISTOL

### Department of Biochemistry

### RESEARCH POSTS IN DNA-PROTEIN INTERACTIONS

Applications are invited for both a Post-Doctoral Research Fellowship and for a Graduate Studentship to work with Dr. S.E. Halford on the molecular mechanisms of DNA recognition by proteins. These include the *EcoRV* restriction enzyme, whose structure has recently been determined and is now available in this laboratory.

The fellowship is funded by the SERC for three years, starting from April 1st (though the start can be delayed for a suitable candidate), with an initial salary at the appropriate point on the RA1A scale (£9,865-£12,150). Prior experience in either molecular biology or protein chemistry/enzymology is desirable but not essential. The Studentship is also funded by the SERC and is available from October 1st. Please obtain further details on Bristol 303136 (answerphone after 5.00 p.m.) or write to Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference A232.

Informal enquiries can be made to Dr. S.E. Halford on Bristol (0272) 303520.

*An Equal Opportunities Employer.*

(8570)A

# International Center for Agricultural Research in the Dry Areas (ICARDA)

## POSITION ANNOUNCEMENT HEAD, SCIENTIFIC INFORMATION SERVICE

ICARDA invites applications for this position at its main station in Aleppo, Syria.

ICARDA is one of the 13 international centers, supported by the Consultative Group on International Agricultural Research (CGIAR), a consortium of donor government's, international agencies and philanthropic foundations. It is concerned with agriculture in those regions that have a hot, dry summer and where cropping must begin in winter, the only season when rain falls. ICARDA has research programs dealing with cereals, food legumes, pasture, forage and livestock, as well as the management of farm resources. The Head Scientific Information Service is responsible for ensuring that the work and achievements of ICARDA are adequately reported to the scientific community and to the donors, and for providing an effective and up-to-date supply of scientific information to the researchers within ICARDA or collaborating with ICARDA.

### FUNCTIONS

The Head Scientific Information Service will report to the Deputy Director General (Research), and will be responsible for planning and supervising all operations of the program, which include public relations and the preparation of general-interest items, such as the Center's Annual Report. The sub-units involve editing and composition typing (English and Arabic), photography, graphic arts, printing, the Center's library, documentation, and specialized information services such as those on lentil, faba bean and cereals.

As the only international agricultural research center located in the region of West Asia and North Africa, ICARDA has special responsibilities to encourage networking and resource-sharing among the national agricultural information programs of the region, and to provide information from the region to meet the needs of CGIAR centers in other parts of the world.

### QUALIFICATIONS

Ideally, candidates will have had graduate training in agricultural science as well as in information science or journalism. All candidates must have held a position, for at least ten years, with management responsibility for the information function in a research environment. They must have extensive knowledge of the international mechanisms for sharing agricultural information and, preferably, will have had experience of working within an international organization and managing staff from a range of different cultures. The over-riding language qualification is for highly effective communication in English, both written and oral, but knowledge of Arabic or French would be an additional advantage.

### APPLICATIONS

Salary, other benefits, and general conditions of service are internationally competitive. Candidates with suitable qualifications are requested to send their applications and the names and addresses of two professional referees quoting Ref. No. STIP/2/88 to the **Deputy Director General (Research), ICARDA, P.O. Box 5466, Aleppo, Syria.**

Applications are accepted up to March 31st, 1989 or until a suitable candidate is selected.

(W5922)A

### UNIVERSITY OF LIVERPOOL Department of Earth Sciences

Applications are invited for two posts of postdoctoral Senior Research Assistant in the Department of Earth Sciences. The posts, tenable for three years from 1st April 1989, entail geological mapping and volcanological research in the Central Fells of the English Lake District. The work, with a team led by Dr. B.P. Kokelaar at Liverpool, and in collaboration with the B.G.S., will be in fulfilment of a NERC/BGS Mapping Contract. Informal enquiries may be made to Dr. Kokelaar. Tel. 051-794 5188.

Initial salary £9,865 per annum.

Applications, together with the names of three referees, should be received not later than 17 March 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3BX**, from whom further particulars may be obtained.

Quote ref. RV/278/N

An Equal Opportunity Employer

(8538)A

### UNIVERSITY OF DURHAM DEPARTMENT OF GEOLOGICAL SCIENCES LECTURER IN BASIN DYNAMICS (NAAS)

Applications are invited for a lectureship in Basin Dynamics, which is expected to be available under the UGC NASS Scheme in the expanding Department of Geological Sciences. Candidates should have proven research experience with a background in sedimentology/stratigraphy/structural geology. Preference will be given to persons with experience of working with or in the oil industry. Excellent opportunities exist for co-operative research in the fields of: palaeo- and neo-tectonics, the geophysics of lithospheric deformation and clastic and carbonate sedimentology.

The appointment may be made initially on either the Lecturer Grade A Scale (£9,260 – £14,500) or on the Grade B Scale (£15,105 – £19,310).

Further particulars may be obtained from the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE (Tel. No. (091) 374 2263)** to whom applications (three copies), including the names of three referees, should be sent not later than **Monday, 3rd April, 1989** (quoting ref. GL1).

(8599)A



## Cruachem

### POSTDOCTORAL CHEMIST Nucleoside/Oligonucleotide Synthesis

Cruachem Ltd is a small but growing company which has built a world-wide reputation in the field of DNA synthesis.

Following our move to purpose-built laboratories at the West of Scotland Science Park on the outskirts of Glasgow, we now require a Postdoctoral Chemist to participate in our active Research and Development effort.

The successful applicant will be involved in a variety of projects, including evaluation and optimisation of the performance of DNA synthesizers and of the methodology of DNA synthesis, laboratory-scale synthesis of nucleoside derivatives and testing of computer software for the control of laboratory instruments.

Ideally, candidates should have some experience of the use of automated DNA synthesizers as well as hands-on experience of the synthesis of nucleoside analogues and derivatives, although candidates with experience in only one of these fields will be considered. Familiarity with the operation of IBM-compatible personal computers would be an advantage, as would a broad understanding of the applications and uses of synthetic oligonucleotides in Molecular Biology and Biochemistry.

In addition to a competitive starting salary, there is a good benefits package, including reimbursement of relocation expenses if necessary.

Please apply in writing, enclosing a comprehensive Curriculum Vitae to:

**Ian Wilkie,  
Chief Executive,  
Cruachem Ltd,  
Todd Campus,  
West of Scotland Science Park,  
Acre Road,  
GLASGOW G20 0UA.**

(8507)A



# THE UNIVERSITY OF BIRMINGHAM

## NEW ACADEMIC APPOINTMENTS SCHEME

Following the initiative by the UGC to create opportunities for young new academic staff, the University expects to be able to offer lectureships in the following subjects for October 1989:

### School of Biochemistry

#### Lecturer in Protein Biophysics (Reference BC 4310)

The appointee will have established expertise in one or more approaches to the study of protein-protein, protein-DNA or protein-ligand interaction. Research interests related or complementary to those already in the School of Biochemistry and/or experience in protein engineering would be an advantage.

### School of Computing and Computer Science

#### Lecturer in Systems Analysis and Design (Reference CS 4311)

Lecturer required to specialise in research, teaching and project supervision primarily in the area of systems analysis and design.

### School of Physics and Space Research

#### Lecturer in Solar and Stellar Physics (Reference PH 4312)

To study the seismology of the Sun and Stars using Doppler spectroscopy and photometric techniques at various observational sites around the world.

#### Lecturer in Environmental and Biomedical Physics (Reference PH 4313)

To study low level dosimetry and to develop biomedical applications of imaging.

#### Lecturer in Chemical Physics (Reference PH 4314)

To investigate reaction rates of ionic atoms and molecules present in upper atmosphere and interstellar plasmas.

Although there is no upper age limit for candidates, it is the intention of the Scheme to enable Universities to recruit younger academic staff. Salary will be within Lecturer A Scale £9,260 – £14,500.

Application forms and further particulars are available from: **The Senior Assistant Registrar (Science), The University of Birmingham, P.O. Box 363, Birmingham, B15 2TT. Applications (three copies) should be returned by 31st March.**

**AN EQUAL OPPORTUNITIES EMPLOYER**

(8582)A

## ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

### Anatomical Society Research Studentships

Applications are invited from Departments of Anatomical Sciences in the UK and Ireland for Research Studentships tenable in the Anatomical Sciences for a period up to three years. Students nominated by Departments must be graduates of British or Irish Universities and will be expected to register for a higher degree. The stipend will be commensurate with basic Research Council rates.

### Anatomical Society Senior Visiting Fellowship

Applications are invited from Overseas Scientists for one Fellowship tenable for a period of less than a year in a Department of Anatomical Sciences in the UK or Ireland. Applicants should be of post-doctoral or comparable status and must have arranged sponsorship in the Department in which they intend to work. Some assistance towards travel and subsistence will be provided.

Further particulars should be obtained from the Acting Secretary, Dr Ian Whitmore, Department of Cell and Structural Biology, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, to whom applications should be sent by 28 April 1989.

(8606)A

## NMR Spectroscopist

### Center for Advanced Research in Biotechnology

The Center for Advanced Research in Biotechnology (CARB) is seeking an NMR spectroscopist to fill a faculty level, tenure track position of the University of Maryland. The successful candidate will be especially interested in using NMR methods to study macromolecular structure and function, and will be expected to build a vigorous research program. The level of the appointment is open, depending on background and experience.

**CARB's Mission:** CARB is joint basic research venture between the University of Maryland and the National Institute of Standards and Technology. (NIST, formerly the National Bureau of Standards). Our goal is to build a center of 27 research excellence in the area of protein structure, function, engineering and design. CARB's new 40,000 Sq. ft. research facility is situated just outside Washington DC, on the newly established Shady Grove Campus of the University of Maryland, and in the heart of Maryland's rapidly growing biotechnology community.

**State-of-the art Labs:** CARB has developed groups in protein crystallography, macromolecular theory and modeling, molecular biology and physical biochemistry, and is now focusing on developing a strong research program in the use of NMR techniques to study protein structure and function. Our labs are equipped with state-of-the art instrumentation, including a minisupercomputer, microvaxs, and several graphics workstations. The University has committed to the purchase of a 500MHz instrument in FY 1989 to enhance the development of CARB's NMR group.

Please send a current CV, an outline of future research interests and goals, a list of individuals who will serve as references, and request that at least three letters of reference be sent to **The CARB NMR Search Committee, CARB, 9600 Gudelsky Drive, Rockville, MD 20850.**

*The University of Maryland is an equal opportunity employer.* (NW3375)A

## THE SCHOOL OF PHARMACY UNIVERSITY OF LONDON

### Fixed-term Lectureship in Pharmaceutical Chemistry

Applications are invited for a fixed-term Lectureship in the Department of Pharmaceutical Chemistry. The appointment is available immediately and is for a period up to and including September 1991. The successful candidate will be required: (i) to teach biochemistry to undergraduate students (ii) to participate in biochemistry practical classes and (iii) to pursue research in the field of receptor biochemistry. Experience in protein chemical methods/antibody production would be an advantage but is not essential.

The salary will be according to the scale for University Lecturers (Grade A £9,260 – £14,500; Grade B £15,105 – £19,130 plus £1,650 London Allowance). Applications in the form of a curriculum vitae and the names of at least two referees should be sent as soon as possible, and by no later than the end of March to **Mr N J Rampley, Assistant Secretary, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX.** (8561)A

## CELL BIOLOGIST/BIOCHEMIST

with a strong interest in breast cancer, to establish a program in growth factor/oncogene research. Selection will be based upon experience, proven ability to develop an independent program as evidenced by current grant support and willingness to interact with established programs in a multidisciplinary approach. Expertise in primary culture of human and rodent cells desirable. Core support for the program will be provided by the Michigan Cancer Foundation (rank commensurate with experience).

Submit curriculum vitae, three references, and summaries of current and future research plans by 1 June 1989 to: **Dr. Gloria Heppner, Michigan Cancer Foundation, 110 East Warren, Detroit, MI 48201.**

*Affirmative Action/Equal Opportunity Employer.*

(NW3383)A



NKK(日本鋼管)は、多角化の一環として  
バイオ開発センターを設置し、経験豊富な人材を求めています。

《募集要項》

■業務内容

医薬品・診断薬・食品などのバイオ事業全般の企画・研究開発

\* 本社・企画部門(東京大手町)

\* 研究開発部門(川崎)

■対象者

年齢：30～45歳。企画または研究に豊富な経験を有する人。

▶ 高度に専門的な研究者も歓迎。

《応募方法》

履歴書(写真貼付)および職務・研究内容の要旨  
(形式自由、2枚程度)を下記宛に郵送してください。

\* 応募の秘密は厳守いたします。

\* 応募書類は返却いたしません。

\* 書類選考のうえ、追って連絡いたします。

〒宛先

〒100 東京都千代田区丸ノ内1-1-2

NKK人事部人事第2室(担当：檀野/中村)

☎(03)217-2055

Fax: (03)214-8402

(W5932)A



Our new name  
marks a new beginning...

# Public Perception for Biotechnology and Seeds

For our Agricultural Division we seek a recently qualified Ph.D. scientist who has the expertise to contribute to the Seeds Subdivision efforts in public perception and external relations regarding the areas of agricultural biotechnology and seed-related issues.

Candidates are expected to have a sound knowledge of molecular biology, plant biology and genetics, as well as the ability to defend viewpoints and debate issues in national and international organisations. Proficiency in written and spoken English and German is a necessity; a knowledge of French would be useful.

Please send your application quoting ref. "Nature 223" to M.A. Vogel, Personnel Department, CIBA-GEIGY Ltd., P.O. Box, CH-4002 Basle.

## CIBA-GEIGY

### BAKER MEDICAL RESEARCH INSTITUTE POST DOCTORAL FELLOW

Applications are invited for the above mentioned position in the Biochemical Pharmacology Laboratory. The successful applicant will join an active group studying the regulation of membrane ion transport processes blood vessels and the causes of cardiovascular hypertrophy in hypertension, using a variety of techniques which include cell culture, intracellular fluorescence spectroscopy and M-RNA analyses.

Applicants should have recently obtained or about to complete a PhD degree in biochemistry, physiology, pharmacology or closely related discipline. Current salary A\$28,000 pa. Applications (including C.V. and two letters of reference) or enquiries, should be addressed to

**Dr. A. Bobik,**  
**Baker Medical Research Institute,**  
**P.O. Box 348,**  
**Prahran, Victoria**  
**3181, Australia.** (W5923)A

### WASHINGTON UNIVERSITY SCHOOL OF MEDICINE ST. LOUIS, MISSOURI DEPARTMENT OF PHARMACOLOGY CHAIRPERSON

Applications and nominations are invited for the position of Chairperson of the Department of Pharmacology. Candidates should be distinguished as scholars and scientists with demonstrated commitment to medical and graduate education.

Curriculum vitae and bibliography may be submitted to:

**M.K. King, M.D.**  
**Dean**  
**Washington University School of Medicine**  
**660 South Euclid — Box 8106**  
**St. Louis, Missouri 63110**

*An Equal Opportunity/Affirmative Action Employer.*  
(NW3309)A

### UNIVERSITY OF EDINBURGH

#### Department of Chemistry Lectureship in Organic Chemistry

Applications are invited for the above Lectureship for which the University has sought funding under the UGC's New Academic Appointments Scheme, with effect from 1st October 1989. Under the terms of the scheme, which is intended to provide permanent appointments for young staff, preference will be given to candidates at an early stage in their careers.

Candidates should have achievement and continuing interest in biological aspects of organic chemistry, and should be prepared to outline their short and medium term research objectives. The person appointed will be expected to undertake a normal level of undergraduate teaching of general organic chemistry.

The initial salary will be on Lecturer A scale (£9,260-£14,500) or, exceptionally on Lecturer B scale (£15,105-£19,310).

Applications, giving full details of career, a list of publications and the names of two academic referees should be submitted to the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS** by 28th April 1989. Please quote reference No. **NAAS 2010.** (8551)A

### THE UNIVERSITY OF THE WEST INDIES

Cave Hill Campus, Barbados

### CENTRE FOR RESOURCE MANAGEMENT AND ENVIRONMENTAL STUDIES

Applications are invited for the following new posts in the Centre for Resource Management and Environmental Studies at the Cave Hill Campus of the University of the West Indies, Barbados, to teach at the postgraduate level in a multi-disciplinary programme leading to Diploma and Masters level certification.

(1) FISHERIES AND RESOURCE MANAGEMENT; (2) COASTAL ZONE MANAGEMENT; (3) RESOURCE ECONOMIST; (4) BIOGEOGRAPHER/PLANNER. The posts at (1), (2) and (3) are at the level of Senior Lecturer/Lecturer. Depending on the qualifications and experience of the applicants two appointments may be made at the level of Senior Lecturer. Appointment to the post at (4) will be at the level of Lecturer. For all four posts proven research ability and capacity to attain grant funding are essential and the ability to teach data management, a knowledge of microcomputer systems and Caribbean or other tropical experience are highly desirable. The successful applicant will be expected to assume duties as soon as possible.

**SALARY SCALES:** SENIOR LECTURER BD\$50,712 × 1644-60,576 × 1776-65,904 (B) × 1776-69,456 p.a. LECTURER: BD\$38,208 × 1644-48,072 (B) × 1644-57,936 p.a. Up to five full economy class passages plus baggage allowance of US\$1200 on appointment and normal termination. Special allowance up to US\$400 for shipment of academic books and teaching/research equipment on appointment. Unfurnished accommodation of 10% of basic salary, or optional housing allowance of 20% of basic salary to staff making own housing arrangements. UWI contribution of equivalent of 10% of basic salary to Superannuation Scheme. Annual Study and Travel Grant for self, spouse and up to three children. Book Grant up to BD\$720 per annum. Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status and the names and addresses of three referees should be sent as soon as possible to the Campus Registrar, University of the West Indies, PO Box 64, Bridgetown, Barbados. The University will send further particulars for these posts to all applicants. These particulars may also be obtained from Appointments (36070), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W5921)



# Senior Research Biologist

## Department of Cellular Science

**From £15k inclusive**

**Greenford, Middlesex**

Glaxo Group Research is an expanding Company dedicated to the research and development of medicines to treat human diseases. As part of our continued expansion, a new vacancy has been created within a section of the Department of Cellular Science, who are working on the pathophysiology of various conditions including cancer, skin diseases, connective tissues and diabetes.

We are looking for a Senior Research Biologist to lead a small team, and provide initiative and innovation in developing models of disease states, as well as maintaining established screening programmes.

Ideally you will have several years' experience, working with *in vivo* models of disease states.

In return you can expect to receive a highly competitive salary (depending on your experience) along with all the benefits you would associate with a major organisation, such as guaranteed bonuses, flexible working hours, and relocation assistance where appropriate.

**Please send a full c.v. or telephone for an application form to: Personnel Officer, Glaxo Group Research, Greenford Road, Greenford, Middx UB6 0HE. Telephone 01-422 3434, ext. 2934, quoting reference no. G89043.**

(8612)A

# Glaxo Group Research



## PLANT-MICROBE MOLECULAR BIOLOGISTS

The Department of Microbiology at the University of Vermont is continuing to expand its research base in molecular biology. Tenure track positions at the assistant and/or associate professor level are available in areas of molecular microbiology relevant to the agricultural sciences. Areas of interest include, but are not limited to, 1) molecular aspects of microbe-plant interactions (including viral, bacterial and fungal pathogens and symbionts) and 2) molecular biology of photosynthetic microorganisms. Applicants' research programs are expected to address questions that relate to gene structure, expression or function. A Ph.D. and postdoctoral experience are required. The Department will be housed in a new Microbiology Center currently under construction. Startup funds and interim renovated space are available. Faculty members hold appointments in both the College of Agriculture and Life Sciences and the College of Medicine. The Department's primary emphasis is on research; teaching responsibilities are to undergraduate, graduate and medical students.

The University of Vermont enrolls approximately 10,000 students and is ranked among the top 'Public Ivies' for its academic excellence and agreeable atmosphere. Scenically located near the Green Mountains and on the shores of Lake Champlain, Burlington offers excellent cultural and recreational opportunities as well as proximity to Montreal and Boston.

Send nominations and applications with curriculum vitae, present and future research plans and at least three letters of reference to:

**Search Committee  
Department of Microbiology, Given Building  
University of Vermont, Burlington, Vt. 05405**

Review of applications will begin immediately and continue until the positions are filled. *An Equal Opportunity/Affirmative Action Employer.* Women and minorities are especially encouraged to apply. (NW3364)A

**Board of the Swiss Federal Institutes of Technology**

**Paul-Scherrer-Institute, Villigen/Würenlingen,  
Switzerland**

## DIRECTOR

Applications are invited for the position of Director of the Paul-Scherrer-Institute (PSI) in Würenlingen/Villigen, Switzerland.

The PSI is a federal multidisciplinary research organization (1000 employees) that conducts research in elementary-particle and nuclear physics, solid-state physics, life science, and energy engineering, both nuclear and non-nuclear. It develops and operates advanced research facilities in support of Swiss universities and international research organizations.

The Director is responsible for the scientific, technical and administrative management of the PSI, and normally assumes auxiliary duties on the faculty of the Swiss Federal Institute of Technology in Zürich or the Swiss Federal Institute of Technology in Lausanne.

Candidates for the position of Director should have a strong record of leadership in either a university or an industrial environment. They should also hold an advanced degree, have demonstrated competence in physical, chemical, biological or engineering research, and be reasonably fluent in German, French and English.

The new Director is expected to assume his or her duties in spring 1990. Applications should be submitted by May 31, 1989, to the **President of the Board of the Swiss Federal Institutes of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland.** (W5915)A



## University College London

### NEW ACADEMIC APPOINTMENTS SCHEME

University College London is seeking 18 young, gifted people to fill lectureships from 1 October 1989 for which funds have been sought from the UGC under the NAAS. Applications are invited in the following areas each of which has a record of eminent achievement in teaching and research:

#### **Earth Remote Sensing**

**Department of Physics & Astronomy**

Global observations of the Earth from satellites represents the biggest growth area in modern space science and is important for environmental as well as research and teaching purposes.

#### **Inorganic Chemistry**

**Department of Chemistry**

For this Lectureship in Chemistry candidates should have special interests in the teaching of inorganic chemistry and in research in any area of inorganic chemistry.

#### **Engineering Sedimentology**

**Department of Geological Sciences**

Applicants should have an interest in the influence of diagenesis on engineering and reservoir characteristics of sedimentary rocks.

#### **Computer Modelling of Cognition**

**Department of Psychology**

Candidates should have an interest in the computational modelling of cognitive processes and will be expected to possess or develop skills in connectionist modelling.

#### **Human Genetics**

**Galton Laboratory, Department of Genetics & Biometry**

Applicants should have a broad training in human genetics, be involved in some aspect of the Human Genome Project and be able to share in both elementary and specialist teaching.

#### **Environmental Design and Engineering**

**Bartlett School of Architecture & Planning**

A Lecturer is required to maintain the strength of this central area of the School.

#### **System Design and Development**

**Centre for Information Technology**

To contribute to teaching and research on the definition, design, development, use and evolution of complex computer based systems in a new Centre formed jointly by the Department of Electrical and Electronic Engineering and the Department of Computer Science.

#### **Chemical Engineering Design**

**Department of Chemical & Biochemical Engineering**

Mainstream chemical engineer with design and/or research experience and interest in C.A.D.

#### **Developmental Neuroscience**

**Department of Anatomy & Developmental Biology**

This appointment is needed to maintain the Department's exceptional strength in this growth area.

#### **Protein Chemistry**

**Department of Biochemistry**

Physical biochemist interested in the relation between sequence and the three-dimensional structure and the function of proteins, including those in membranes.

#### **Non-Clinical Chemical Pathology**

**Department of Chemical Pathology**

Non-clinical Lecturer required with a higher qualification and experience in the discipline to conduct research relevant to existing interests in the department and to share in undergraduate and postgraduate teaching.

The salaries will be within Lecturer A, £10,910 - £16,150 inclusive. Further particulars for each appointment from Jacquie Worrall, Telephone: 01-380 7102. Applications, including C.V. and the names and addresses of three referees by **20 March 1989** to the Assistant Secretary (Personnel), UCL, Gower Street, London WC1E 6BT. UCL is an Equal Opportunities Employer.

(8607)A

### **MOLECULAR TUMOR BIOLOGY**

#### **A POSTDOCTORAL/RESEARCH ASSOCIATE POSITION**

is available immediately for candidate with background in one or more of the following areas: Molecular biology, Genetic engineering, Radiation biology, Biochemistry, Virology or Immunology. Candidate will participate in research on the molecular basis of radiation-resistant human cancers with special focus on PCR-based evaluation and comparison of the oncogene sequence(s) in normal vs. tumor cells.

Please send curriculum vitae to: **Dr Anatoly Dritschilo, Professor and Chairman, Department of Radiation Medicine, The Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, 3800 Reservoir Road, N.W., Washington, D.C. 20007.**

Equal Opportunity/Affirmative Action Employer.

(NW3358)A

### **ASSISTANT PROFESSOR**

#### **Haverford College**

Haverford College, 2-year position as Assistant Professor to teach molecular and biochemical biology, immunofluorescence or electron microscopy, and direct student research.

Contact: A. G. Loewy, Haverford College, Haverford, PA 19041. Deadline for applications March 23.

Equal Opportunity Employer/Affirmative Action. (NW3403)A

### **THE UNIVERSITY OF TASMANIA**

#### **INSTITUTE OF ANTARCTIC AND SOUTHERN OCEAN STUDIES (IASOS)**

Applications are invited from suitably qualified men and women for the following position.

#### **ODP SUPERVISING SCIENTIST** (Ref. 41/89)

Australia has become a joint member with Canada of the Ocean Drilling Program, and the Australian ODP Council has nominated the University of Tasmania as the site for the Australian ODP Secretariat for the next three years. The ODP Secretariat will be attached to the University's Institute of Antarctic and Southern Ocean Studies.

The ODP Council seeks a geoscientist, to be appointed at a senior level, as ODP Supervising Scientist. The successful applicant will be primarily responsible for the day-to-day management of Australia's involvement in ODP, will participate in the planning of its scientific program, and will also have research and some teaching responsibilities in the University's Department of Geology, which has vigorous research groups concerned with ocean floor magmatism and geochemistry, and with marine geological studies of the SW Pacific and NE Indian Oceans. Applicants in the field of marine geophysics will be particularly welcome.

The position will be funded for three years at the first instance, at the Reader level, currently \$53,670 per year. Applications should include a complete resume with the names of three referees, and a statement of current research interests.

Further information may be obtained from Professor D.H. Green (tel. 61.02.202477), Dr. R. Varne (tel. 61.02.202468) and by writing (Fax 61.02.202186) to The Director, Institute of Antarctic and Southern Ocean Studies, the University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania 7001, Australia.

Position information and application forms available from the Staff Office Secretary, tel. (61.02) 202013. Applications quoting ref. should be sent to the Staff Officer, University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania 7001, Australia, by 31 March 1989.

The University is an Equal Opportunity Employer (W5918)A

**nature**  
— the professionals' choice



**At the Swiss Federal Institute of  
Technology in Zurich the chair of  
FOOD MICROBIOLOGY  
is to be filled.**

Duties of the new professor include teaching and research in the general field of food microbiology. Applicants will have a university degree in food science, food microbiology or microbiology and several years of successful independent research and development work in food microbiology.

Enthusiasm to teach at all university levels and willingness to interact with colleagues within and outside the university are indispensable.

Candidates should address their application with curriculum vitae and list of publications no later than **April 30, 1989**, to the **President of ETH Zurich, Prof. Dr. Hans Bühlmann, ETH-Zentrum, CH-8092 Zurich. (W5917)A**

SYNTHELABO RECHERCHE (L.E.R.S.)

**Head of the Cerebral Circulation  
& Metabolism Group**  
**Biology Department**

SYNTHELABO RECHERCHE (L.E.R.S.) is the Division responsible for research and development in this leading French pharmaceutical company with rapidly expanding international interests.

The position involves the scientific direction of a group of about 15 scientific staff with opportunities for undertaking original research within a scientifically stimulating environment in well-equipped and newly built laboratories situated 5 km from the centre of Paris.

The successful candidate should have several years post-doctoral experience and have made a substantial contribution in the fields of cerebrovascular and neuroprotective research.

Experience in the pharmaceutical industry and a basic knowledge of French would be useful but not essential.

Please write, in confidence, with a detailed curriculum vitae to:  
M. Campionnet, Human Resources Department  
SYNTHELABO RECHERCHE (L.E.R.S.)  
58 rue de la Glacière 75013 Paris, France.

**SYNTHELABO RECHERCHE**  
(L.E.R.S.)

(W5934)A



L.R.W.

**Senior Appointment in  
Research Management**

The Medical Research Council wishes to appoint an experienced scientist or medical practitioner to the staff of its headquarters office in Central London, to play a major part in new arrangements for the central management of research funding. Reporting to the Head of Medical Division, and through him to the Secretary and Second Secretary of the Council, the new appointment would have two main areas of responsibility:

■ the development and implementation of policy on a wide range of research topics which cross the boundaries of the MRC's individual research boards. Initially, this will include substantial involvement with the scientific planning of the New National Centre for Multispeciality Postgraduate Medical Education and Research which the Council is proposing to set up at Hammersmith in partnership with the Royal Postgraduate Medical School.

■ management of the headquarters office Grants and Training Awards Group, which administers the Council's grants and training awards schemes for the support of research and research workers in universities, polytechnics, hospitals and independent research institutes. The person appointed will be expected to play a major role in reviewing and developing these scheme and in liaising with higher education and research institutions.

The post is likely to be of interest to scientists with a substantial track record in biomedical research together with significant managerial experience. The Council would be particularly interested to hear from clinically-qualified candidates but non-clinical scientists are equally encouraged to apply.

Appointments to the Council's headquarters staff are usually for a probationary period at the end of which they may be made permanent. In this case, the Council would also be prepared to consider a fixed term contract for someone interested in secondment from their present employer. The salary payable to non-clinical staff is on Civil Service Grade 6, £21,633-£32,826 (plus London Weighting £1,750 p.a.) There is a separate salary scale for clinically-qualified candidates rising to £36,786 (plus London Weighting £1,750). In addition, a 4½% supplement is payable to members of the MRC Pension Scheme.

**Application forms and further information about the post are available by telephoning or by writing to Miss Kathryn Fenn in the Headquarters Office Staffing Group, Medical Research Council, 20 Park Crescent, London W1N 4AL (01-636 5422 x 404).**

**the closing date for applications is 10 April 1989. Reg. No. 185725 VAT No. 238 6105 65**

**MRC**

Medical Research Council

(8583)A

**UNIVERSITY OF CAMBRIDGE  
DEPARTMENT OF EARTH SCIENCES  
ASSISTANT DIRECTORSHIP OF RESEARCH  
IN MARINE GEOPHYSICS**

Applications invited for Assistant Directorship of Research from workers with experience of proposing, organising and running research cruises, and some involvement in instrument development in any area of marine geophysics.

Salary scale £12,760-£19,310.

Further particulars from the **Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ**, to whom applications, with a c.v. (10 copies), and names of three referees, should be sent by **2 May, 1989.**

(8548)A



## MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

Food Science Division  
London

### Head of Food Microbiology Branch

You will be responsible for the day-to-day and long-term administration of the Branch. You will generate and present scientific and technical advice on food microbiology and represent the Ministry at both national and international meetings.

You should have a good degree in microbiology with extensive post-graduate experience in food microbiology, preferably with experience of research and the food industry.

Starting salary will be in the range £18,440-£23,485 (including £1750 Inner London Weighting) with further increments, depending on performance, up to £27,670. Ref: S(E)677

### Food Microbiologist

You will deputise for the Head of the Food Microbiology Branch and assist him in the generation and presentation of scientific and technical advice on food microbiology. You will provide briefing to senior members of staff who attend national and international meetings and you may stand in for the Head of Branch at these meetings. You will also be involved in the co-ordination of the scientific aspects of MAFF's activities in relation to food poisoning incidents.

You should have a good degree in microbiology with at least 4 years' relevant post-graduate experience, preferably with experience of research and the food industry.

Starting salary will be in the range £14,690-£18,780 (including £1750 Inner London Weighting) with further increments, depending on performance, up to £21,675. Ref: SB/12/AE

RELOCATION EXPENSES UP TO £5000 MAY BE AVAILABLE FOR BOTH POSTS.

For further details and an application form (to be returned by 17 March 1989) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours). Please quote appropriate reference.

The Civil Service is an equal opportunity employer

*Scientific*  
**CIVIL SERVICE**

(8614)A

**nature** — the professionals'  
choice



### A F B – Arzneimittelforschung GmbH in Berlin

AFB – Arzneimittelforschung Berlin GmbH in Berlin is a drug research company rapidly growing both nationally and internationally.

In the Department of Biometry and Statistics we are looking for a

### Senior Biostatistician

to work in our Head Office in Berlin (West).

The ideal candidate has a degree in Mathematics or Statistics from a recognized University and at least five years working experience in the pharmaceutical industry or in a medical research or health institution. Apart from a thorough knowledge of statistical methodology, he or she should be familiar with statistical software packages such as SAS and SPSS and their application on main frames and PC networks. Experience is also required in the preparation of technical reports (in English). A working knowledge of German is desirable or at least the willingness to learn German quickly. We offer a competitive salary commensurate to experience, and excellent opportunities for career development.

Please send your application to the **personnel manager**.

**A F B – Arzneimittelforschung GmbH in Berlin**  
**Kurfürstendamm 217, D-1000 Berlin 15**

(W5925)A

### UNIVERSITY OF BIRMINGHAM SCHOOL OF BIOLOGICAL SCIENCES

#### Temporary Lectureship (Ref: BS2005)

A position is available for three years for a Temporary Lecturer to work in collaboration with the group of Professor Nigel Brown in the new School of Biological Sciences (the former Departments of Genetics, Microbiology, Plant Biology and Zoology). Preference will be given to applicants wishing to work on the mechanisms of heavy metal metabolism and resistance in microorganisms, but the overriding criterion will be academic excellence. The successful applicant will have opportunity to develop independent research programmes. The appointment will be on the Lecturer 'A/B' scale, £9,260-£19,310, plus super-annuation.

For an informal discussion on the position, please contact Professor N L Brown, (telephone 021-414 6556, Fax 021-414 5925).

For further particulars of the above post and an application form, telephone 021-414 6383 quoting reference number BS2005. Applications (3 copies) should be sent to the **Senior Assistant Registrar, Faculty of Science, PO Box 363, Birmingham B15 2TT** by 21st April 1989.

An Equal Opportunities Employer

(8581)A

### UNIVERSITY OF CAMBRIDGE DEPARTMENT OF EARTH SCIENCES UNIVERSITY LECTURERS OR ASSISTANT LECTURERS IN ENVIRONMENTAL CHANGE

These will be joint appointments with the Sub-Department of Quaternary Research.

For one of the posts, preference will be given to applicants working on problems of Recent Earth (including ocean) history or behavior, using or developing physical, chemical, isotopic or geochronological methods.

For the other, preference will be given to applicants working on microfossils in palaeoenvironmental, evolutionary or bio-chronological studies.

Appointment at Lecturer or Assistant Lecturer level, according to age. Lecturer salary scale £13,365 – £20,626. Assistant Lecturer salary scale £10,460 – £14,500.

Further particulars from the **Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ**, to whom applications, with a c.v. (10 copies), and names of three referees, should be sent by **2nd May, 1989**. (8554)A

# Protein Crystallographer

There is a vacancy for a protein crystallographer in a newly formed Drug Design Group in the Pharmaceutical Division at Sandoz.

We are committed to an extensive basic research program involving X-ray structure analyses of medicinally/pharmacologically important proteins and protein drug complexes. X-ray equipment will include an area detector, rotating anode generator and computer graphics. There is also close collaboration with NMR and molecular modelling groups, providing an ideal environment for joint drug design projects.

Applicants should have a PhD and experience in most aspects of protein crystallography.

*If you are interested please send your application with a detailed C.V. to:*

**Sandoz Ltd., Personnel Department, ref. 8138,  
attention Dr. R. Racine  
CH-4002 Basle, Switzerland**



(W5914)A

UNIVERSITY OF EXETER (U.K.)  
UNIVERSITAET MARBURG (F.R.G.)  
INSTITUT JACQUES MONOD (PARIS)  
**POST-DOCTORAL CHEMISTS/  
BIOCHEMISTS/MOLECULAR  
BIOLOGISTS**

Applications are sought from chemists, biochemists and molecular biologists for 3 post-doctoral vacancies funded by a major collaborative grant under the European Community's SCIENCE Programme for 3 years from 1 April 1989, or as soon as possible thereafter. The project concerns biochemical characterization of insect steroid (ecdysteroid) hormone receptors and the receptor gene. The collaborating groups are those of Professor J. Koolman (Marburg), Dr. J.-A. Lepesant (Paris) and Dr. L. Dinan (Exeter). One vacancy exists at each centre, with the vacancies in Exeter and Marburg being suitable for applicants with a background in chemistry and/or biochemistry and the Paris vacancy appropriate for a molecular biologist. Previous research experience of steroid hormones, receptors, monoclonal antibody production, modern protein purification methods or molecular biological techniques advantageous. Applicants for each post must be nationals of another European Community country other than the one in which the research centre is located. Salaries will be on the national scale appropriate for the country involved. Travel funds are available for research visits and discussion meetings between the collaborating laboratories.

Applications (3 copies), including details of previous experience, a list of publications, the names of 3 referees and stating which post(s) you wish to be considered for, should be sent to **Dr. L. Dinan, Department of Biological Sciences, University of Exeter, Perry Road, Exeter, Devon EX4 4QG, U.K.**, within four weeks of the appearance of this advertisement. It would be helpful if applicants could also send copies of the publications which they regard as most representative of their research to date. Further particulars obtainable from Dr. Dinan (Tel: (0392) 264605/264603, Fax: (0392) 263108, Telex: 42894 EXUNIV G). (8493)A

## Faculty Positions in X-ray Crystallography

The Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School and the Dana-Farber Cancer Institute are seeking applications to fill two positions at the **Assistant Professor** level to be located at the Dana-Farber Cancer Institute. Applicants should be interested in using crystallographic methods to solve problems in Structural Biology and Biochemistry and should have an appropriate combination of related graduate and postdoctoral level research experience. Interested applicants should send an outline of future research plans, a curriculum vitae, bibliography and the names of three to four references to: **Professor Christopher Walsh, Chairman, Biological Chemistry and Molecular Pharmacology, Search Committee, Harvard Medical School, 240 Longwood Avenue, Room C1-213, Boston, MA 02115. Women and minorities are encouraged to apply. Harvard University and Dana-Farber Cancer Institute are Equal Opportunity Employers.** (NW3386)A

## POSTDOCTORAL POSITION IN MOLECULAR BIOLOGY UNIVERSITY OF CALIFORNIA, SAN DIEGO

available to study a novel system of regulation of gene expression and DNA replication by silicon in a lower eukaryote, by analysis of gene banks. Cloned yeast genes have been used successfully to identify diatom genes of similar function and at least one yeast mutation *leu 2<sup>-</sup>* has been complemented using cloned diatom DNA sequences. Background in molecular biology. Proficiency in recombinant DNA techniques: cDNA cloning, analysis of gene banks, vector construction, Southern and Northern hybridization, transformation, analysis of regulatory sequences, DNA sequencing. Salary starting at \$25,332 to \$27,624, depending on qualification. Send detailed CV and three names for reference to: **Dr. B. E. Volcani, Scripps Institution of Oceanography, University of California, La Jolla, CA 92093, tel (619) 534-2194.** Applications will be accepted until positions are filled. *An Equal Opportunity/Affirmative Action Employer.* (NW3396)A

# ENTOMOLOGIST BIOLOGICAL PEST CONTROL

Chr. Hansen's Bio Systems A/S urgently seek an entomologist for our Biological Pest Control Section, Biopax. The position involves support for technical/sales functions in dealing with the production and practical application of beneficial insects and micro-biological control products.

**We are involved** in the development, propagation and marketing of biological products, the bulk of which are sold in Scandinavia.

Applicants should have a degree in entomology or ecology, preferably with experience of research in the use of beneficial insects and population biology, combined with either practical experience of horticulture or a good understanding of horticulture practice. In selecting suitable candidates we shall place emphasis on analytical skills and project management experience.

The position involves close cooperation with technical services/sales, marketing, product development and production. The ability to form good working relationships with colleagues is essential.

**We are seeking** a dynamic person who can deal with production and with further development of existing products, and who can initiate research into new products.

If, and when, time permits, additional short or long term projects will be added to the job function. In the longer term it is expected that the job function will change in a more development oriented direction.

The successful applicant will be based at Karlebo and/or at Hørsholm.

For further information please write or phone to Mr. Aage Nyholm Thomsen, Managing Director of Chr. Hansen's Bio Systems A/S, 10-12 Bøge Allé, DK-2970 Hørsholm, phone 02 76 66 66, ext. 4107.

Applicants should send a curriculum vitae to the Personnel Department, Chr. Hansen's Laboratorium A/S, 10-12 Bøge Allé, DK-2970 Hørsholm.

**CHR. HANSEN'S bio  
systems**

Chr. Hansen Bio System A/S is a subsidiary of Chr. Hansen's Laboratorium A/S, which is a Danish, internationally working group with more than 900 employees. For more than 100 years Chr. Hansen's Laboratorium A/S has sold special products and ingredients to the food industry. Chr. Hansen Bio System A/S is involved in marketing of biological products to improve the productivity within the agriculture sector.

(W5931)A



**KLINIKUM DER  
ALBERT-LUDWIGS-  
UNIVERSITÄT FREIBURG**

Applications are invited for the position of an

## independent university assistant (C1)

at the Institute for Biology III (Dept. Genetics), starting October 1989 (approx. for 5 years).

The candidate is expected to head a small research group and has the possibility for habilitation. His research should center around molecular problems in cell, developmental, or tumour biology.

Applications incl. CV, list of publications, research proposal and two letters of reference, at the latest March 31, to Prof. A.E. Sippel, ZMBH, INF 282, D-6900 Heidelberg, FRG. (W5926)A

**MRC**  
Medical Research Council

## MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH IMMUNOLOGY DIVISION

Applications are invited for a three year postdoctoral position in the Division of Immunology on control of B cell activation, proliferation and differentiation. The work will involve biological biochemical and molecular biological approaches. Previous relevant experience, although advantageous, is not essential.

Salary will be in the range £12,520 – £15,950 inclusive of London Allowance per annum.

Please send applications, including covering letter and full CV together with the names of two referees, to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA. Closing date for this post will be 24 March 1989. Please quote job reference: 0521/II.**

The MRC is an Equal Opportunities Employer. (8584)A

## MASSEY UNIVERSITY

Palmerston North, New Zealand

### DIRECTOR, IMAGE ANALYSIS UNIT

Applications are invited from both men and women for the above appointment which includes half-time lecturing in the Department of Physics and Biophysics. The Unit undertakes fundamental research into image analysis methodology and provides a client advisory service. The appointment is for an initial period of three years.

The appointee would be expected to contribute to Electronics teaching in the Department of Physics and Biophysics. A practical knowledge and experience of DEC's VMS operating system, PC-DOS for the IBM-PC AT, "C" programming language and of general image analysis methodology is required. An ability to interact well with users is essential. Previous relevant management experience would be an advantage.

Salary is in the range NZ\$35,000 to NZ\$42,500 pa

Further information is available from Professor P T Callaghan, Head of Department of Physics/Biophysics (PN 69 099 extn 8364) and Professor D A D Parry, Chairman, Image Analysis Advisory Committee (extn 7473).

Applications including the names and addresses of three referees who may be contacted should be forwarded to Mrs V B Bretherton, Personnel Section, Massey University, Palmerston North, New Zealand, before 30 April 1989. Applicants resident in the UK should also send a copy to Appointments (36039), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, from whom further information is also available.

(W5912)A

## UNIVERSITY OF EDINBURGH

### POSTDOCTORAL RESEARCH FELLOW (BIOCHEMISTRY)

#### DEPARTMENT OF VETERINARY CLINICAL STUDIES

Applications are invited for a three-year, extramurally funded research post involving mast cell proteinases, their isolation, characterisation and functional properties.

The post is available from 1st April 1989. Salary will be on the Research 1A scale (£9,865-£11,680) with placement according to age, qualifications and experience.

Further particulars may be obtained from the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS**, with whom applications, including a curriculum vitae and the names and addresses of two referees, should be lodged not later than 22 March 1989.

Please quote reference no. 5630. (8576)A

## POSTDOCTORAL POSITION

available to study the biological and biochemical consequences of enhanced oncogene expression in human neuroblastoma cells. Project involves purification and characterization of autocrine growth factors. Experience in peptide biochemistry and/or molecular biology of preferred. Position is paid according to BAT IIa (DM 74,000/£22,000 p.a.).

Applicants should send a CV, including publications and the names of two referees to **Dr. Lothar Schweigerer, Dept. of Hematology/Oncology, Children's Hospital, University of Heidelberg, INF 150, 6900 Heidelberg, FRG.** (W5928)A



# Molecular Graphics

## Up to £23,000

The Sunbury Research Centre enjoys a worldwide reputation for technical excellence and provides a stimulating environment for ambitious research professionals. Our scientists and engineers enjoy the facilities of one of the most advanced and best equipped research establishments in Europe.

The BP Group has extensive interests in developing new materials for applications areas, including polymer composites, membranes, speciality chemicals and catalysts where molecular graphics and computer modelling techniques are playing a fundamental role in focused research. This has created challenging opportunities for exceptional applications-orientated chemists to research into the structure/property relationships of molecules and materials.

You will have a good honours degree and PhD in Chemistry, Chemical Physics or Material Sciences with a sound theoretical knowledge of polymer physics or chemistry. Exposure to computational techniques, theoretical chemistry and chemical crystallography would be desirable.

In addition to excellent career prospects you can expect an attractive salary and first-class benefits package.

**Please telephone or write for an application form, quoting ref P206/1/N to: The Recruitment Officer, BP Research, Sunbury Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN. Tel: 0932 762028.**

*BP is an equal opportunity employer.*



## BP RESEARCH

### Sunbury Research Centre

*...creating tomorrow*

(8613)A

#### NOTTINGHAM HEALTH AUTHORITY CITY HOSPITAL

#### PRINCIPAL MOLECULAR GENETICIST

The Trent Sub-Regional Genetic Service based in the above hospital, has a vacancy for an experienced Molecular Geneticist at Principal Grade to help provide and develop laboratory support for this established clinical specialty serving 1.5 million population. There is a second senior molecular geneticist in post. A Professor of Medical Genetics is to be appointed to direct the medical genetics service with its integrated molecular and cytogenetic laboratories during 1989, to be followed by the appointment of a Senior Lecturer in Molecular Genetics early in 1990.

For further information contact Dr J S Fitzsimmons, Consultant Clinical Geneticist, tel: (0602) 691169, ext 2944.

Salary: £15,180 to £20,989 p.a.

Application form and job description from the Personnel Department, Unit Headquarters, City Hospital, Hucknall Road, Nottingham NG5 1PB Tel: 625459 (24 hour answering service).

Closing date: 17th March 1989.  
(8553)A

#### University of Nottingham MEDICAL SCHOOL LECTURESHIPS IN BIOCHEMISTRY

Applications are invited for two posts of Lecturer in the modern well-equipped Department of Biochemistry located in The Queen's Medical Centre, Nottingham. The Department is responsible for teaching Biochemistry to medical and science students to honours level. Candidates should provide evidence of an active research interest in biochemical aspects of mammalian biology or medicine including in the case of one of the posts experience in cell biological or molecular biological techniques.

Salary will depend on the non-clinical Lecturer scale and the appointment will carry membership of USS. The initial salary will depend on qualifications, age and experience.

Professor F W Hemming will be pleased to answer telephone enquiries about the appointments (tel: 0602 421421 ext. 3619).

Other particulars and a form of application may be obtained from the **Deputy Registrar and Secretary, Medical School, Queen's Medical Centre, Nottingham NG7 2UH to whom completed applications should be returned by 31 March 1989.**

(8557)A

#### University of Bristol New Academic Appointment Scheme Department of Zoology

#### Lectureship in Behavioural and/or Population Ecology

This appointment forms part of an expansion of behavioural and population ecology in the Department. The successful candidate will, while developing his/her own interests, be expected to establish collaborative links with our research groups working in fields ranging from mammalian and avian to estuarine and fish ecology, and to take part in an expanded teaching programme in behavioural and population ecology. The post will attract substantial setting up funds.

Salary will be assessed in the Lecturer Grade A scale £9,260-£14,500 per annum.

For further details telephone Bristol 303136 (ansaphone after 5pm) or write to the **Personnel Office, Senate House, Bristol BS8 1TH.** Please quote reference JPB31.

Closing date 20 March 1989.

*An Equal Opportunities Employer*

(8571)A

#### UNIVERSITY OF CAMBRIDGE

#### University Lecturer in the Department of Anatomy

Applications are invited from suitably qualified graduates, including applicants from abroad, for the post of a University Lecturer in the Department of Anatomy to hold office from 1 October 1989 or as soon thereafter as possible. The Department has extensive and well-funded facilities for research, in various branches of neuroscience including brain research, neuroendocrinology and behavioural studies, molecular, cellular and developmental biology.

Salary range: £13,365 to £20,616.

Further information and application form from **The Secretary, Appointments Committee, 19 Trumpington Street, Cambridge, CB2 1QA** (Tel: 0223 33499 Fax: 0223 332355).

Closing date: 8 April 1989.

(8601)A

# UNIVERSITY OF OXFORD



## DEPARTMENT OF PLANT SCIENCES SIBTHORPIAN PROFESSORSHIP OF RURAL ECONOMY (PLANT SCIENCE)

The electors intend to proceed to an election to the Sibthorpian Professorship of Rural Economy (Plant Science) from 1 October 1989 or such later date as may be arranged. On appointment the professor will be required to assume the duties of Head of the Department of Plant Sciences not later than 1 October 1991. The stipend of the professor is at present £26,905 a year. There will also be pensionable allowance, currently £3,585, as head of department.

**Applications (eight copies or one from overseas candidates), naming three referees, but without testimonials, should be received not later than 13 March 1989 by the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, from whom further particulars may be obtained.**

## University Lectureship in Metamorphic Petrology in association with St. Cross College

Applications are invited for a lectureship, to be filled from 1st October 1989 or as soon as possible thereafter, within the field of metamorphic petrology. The lecturer will be responsible for teaching metamorphic petrology, and may have research interests in any area within this subject. Stipend will be according to age on the scale £9,865 to £20,615 p.a., together with membership of the University's Superannuation Scheme. The lectureship, open to men and women, will be held in conjunction with an Official Fellowship at St. Cross College.

Further particulars may be obtained from Professor J. F. Dewey, Department of Earth Sciences, Parks Road, Oxford OX1 3PR to whom application (four copies, or one in the case of overseas applicants) including curriculum vitae, a statement of experience and research interests, a list of publications, and the names and addresses of three referees should be sent so as to arrive before March 30th 1989.

## OXFORD CENTRE FOR MOLECULAR SCIENCES Postdoctoral Research Assistant Time resolved studies on enzyme catalysis

The research assistant will work jointly with Dr. J. Hajdu, Dr. L. N. Johnson and Dr. A. J. Pratt on the application of Laue diffraction methods for the study of reaction mechanisms in enzyme crystals. In the first instance, the work will relate to glycogen phosphorylase and the design of suitable caged substrates in order to achieve synchronisation of the start of reaction with the start of data collection.

The post is tenable for 2 years initially, with the possibility of extension, on the Scale RS1A £9865-£12760 (according to age and experience).

**Applications in writing with full CV and 2 referees should be made to The Administrator, Oxford Centre for Molecular Sciences, c/o Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY, from whom further details of the post can be obtained.**

Closing date: 24 March 1989.

(8594)A

*The University is an Equal Opportunity Employer*

## UNIVERSITY COLLEGE LONDON LECTURER IN CHEMISTRY

Applications are invited for a Lectureship in the Department of Chemistry from 1 October 1989. Candidates should have special interests in the teaching of physical chemistry and in research in some area of physical chemistry. It is expected that the appointment will be made within the lower part of the Lecturer salary scale (£9,260 to £14,500 plus £1650 London Allowance).

Further particulars can be obtained from the **Head of the Department of Chemistry University College London, 20 Gordon Street, London WC1H 0AJ or telephone 01-387 7050 ext. 7451.** Applications (6 copies if possible) should reach him on or before 7 April 1989. *Equal Opportunities Employer.*

(8567)A

University of London  
BRITISH POSTGRADUATE MEDICAL FEDERATION



INSTITUTE OF DENTAL SURGERY

## Chair of Oral Biology

The Senate invite applications for the newly-established Chair of Oral Biology. The successful applicant will head the newly formed department which, with reorganisation of the research of the institute, will include a substantial proportion of existing biological work.

The major remit of the Professor will be to initiate an innovative programme of biological research, making best use of the excellent facilities of the Institute and associated Eastman Dental Hospital, and linking as appropriate with other science groups within and outside the BPMF. The Institute has a strong educational programme in which the staff of the department would be expected to participate.

The Institute, which has a particular interest in connective tissue research, wishes to strengthen work in basic science with particular emphasis on cell and molecular biology.

Applications (11 copies) should be submitted to the Teachers' Section (N), University of London, Senate House, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained.

The closing date for receipt of applications is 10 April 1989.

(8600)A

## JUSTUS-LIEBIG- UNIVERSITÄT GIESSEN

Am Institut für Genetik des Fachbereichs Biologie ist sofort die

### C4-Professur für Genetik

(Nachfolge Prof. Dr. Anders)

wieder zu besetzen. Sie sollten auf einem aktuellen Gebiet der Genetik von Entwicklungsprozessen ausgewiesen sein. Mitarbeit im Sonderforschungsbereich "Molekulare Grundlagen zellbiologischer Schaltvorgänge" ist erwünscht. In der Lehre sollen für Studierende der Biologie und benachbarter Fachbereiche klassische und molekulare Genetik vertreten werden.

Die Justus-Liebig-Universität Gießen strebt einen höheren Anteil von Frauen im Wissenschaftsbereich an; deshalb bitten wir qualifizierte Wissenschaftlerinnen nachdrücklich, sich zu bewerben. — Sofern Sie an einer Bewerbung interessiert sind, ist es empfehlenswert, bei uns ein Merkblatt anzufordern, das Sie über die gesetzlichen Einstellungs Voraussetzungen und die erforderlichen Bewerbungsunterlagen informiert. Ihre Bewerbung richten Sie bitte unter Angabe des Aktenzeichens B - 04 bis zum 5. Mai 1989 an den **Präsidenten der Justus-Liebig-Universität Gießen, Postfach 11 14 40, D-6300 Gießen.**

(W5924)A

## UNIVERSITY OF LEICESTER DEPARTMENT OF PHYSIOLOGY POST-DOCTORAL RESEARCH ASSOCIATE MECHANISM AND REGULATION OF THE PLASMA-MEMBRANE CALCIUM PUMP

Applications are invited for the above post. The successful candidate will investigate the sequence and association of biochemical and transport steps of the purified Ca, Mg-ATPase and will also study the regulatory effect of other membrane-bound enzymes on Ca<sup>2+</sup> transport by the pump. The experimental methods include membrane-enzyme and antibody purification, affinity chromatography, SDS-PAGE, and the measurement of Ca<sup>2+</sup> fluxes, ATPase activity and isotopic exchanges with the reconstituted pump. The position is available for three years, is supported by a grant from the Wellcome Trust and involves a collaboration with Aarhus University. The starting date will be 1 May 1989 or as soon as possible thereafter. The salary is in the 1A range £9,865 to £15,720.

Applications, including the names of two referees, should be sent to **Dr. J. D. Cavieres, Department of Physiology, University of Leicester, Leicester LE1 7RH, U.K. (telephone (0533) 523091),** who will be pleased to supply further information. Closing date: 3 April 1989.

(8544)A

# Molecular Biologist

**£13,500-£16,000  
Beckenham, Kent**

- A World leader in Biotechnology
- Novel therapeutic proteins and vaccines
- Established in 1982
- A subsidiary of the Wellcome Foundation Ltd.
- An Equal Opportunity Employer

Wellcome Biotech is at the forefront of the application of Biotechnology to the production of new medical products and we are currently looking for a recently qualified PhD scientist to work in the Eukaryotic expression group within the Molecular Biology Department.

As part of this enthusiastic group, you will be working on the development of systems for high level expression of recombinant genes in mammalian and insect cells. Experience in standard molecular biology techniques is essential, whilst some familiarity with tissue culture is desirable.

In addition to an attractive salary, you can look forward to an extensive range of benefits which include 5 weeks' holiday, pension scheme, profit share scheme, first-class sports and social facilities and a subsidised restaurant.

**If you are interested in this position please write for an application form, to Andrew Wright, Personnel Officer, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, quoting reference 91/62.**

**WELLCOME BIOTECH**



**Wellcome**

(8611)A

## POSTDOCTORAL ASSOCIATE

Position available immediately in the area of peptide synthesis related to the development of novel AIDS vaccines. Position requires individual to participate in the design and synthesis of peptides which mimic anti-receptor antibody variable region structures to be used for the induction of anti-HIV responses in animals. This position is under the direction of Dr. Patrick Kanda, within our Department of Virology and Immunology. Southwest Foundation is a private non-profit institution funded, in part, by NIH. Annual postdoctoral salaries range upward from \$21,070, with consideration for prior, relevant postdoctoral experience. Applicants should have a background in biochemistry and/or organic chemistry or related area, with an interest in immunology.

Please send curriculum vitae and names and telephone numbers of three references to **Personnel Director (888), Southwest Foundation for Biomedical Research, P.O. Box 28147, San Antonio, Texas 78284.**

*Equal opportunity employer m/f.*

(NW3348)A

## ASSISTANT PROFESSOR THE UNIVERSITY OF WISCONSIN - MADISON

The School of Pharmacy at the University of Wisconsin - Madison invites applications for a full-time tenure-track position at the assistant professor level in the area of pharmaceuticals. Candidates should be capable of developing an independent, aggressive, and fundamental program of research on some aspect of the physical chemistry of biomolecules (preferably macromolecules). The primary interest should be in generating new knowledge at the molecular level, though the research should have potential application to drugs. Candidates should hold the Ph.D. in physical, analytical, or biophysical chemistry or in pharmaceuticals, and have relevant postdoctoral research experience. Teaching responsibilities will include undergraduate and graduate courses. Please send curriculum vitae with a description of research interests and names of three references by 1 April 1989 to: **Dr. Kenneth A. Connors, School of Pharmacy, University of Wisconsin, Madison, WI 53706; telephone: 608-262-2990.**

*The University of Wisconsin is an Equal Opportunity/Affirmative Action Employer.*

(NW3393)A

## UNIVERSITY OF GLASGOW DEPARTMENT OF NEUROLOGY

### Postdoctoral Research Fellow—Immunology

Postdoctoral research fellow with extensive experience in cellular immunology required to join newly formed large research group in neurobiology. The main interest of the group is in the altered responses to viruses in post-viral neurological diseases. Salary within £9,865-£15,720 p.a. on Research and Analogous Staff 1A scale. Post available for up to 5 years.

### Two Research Assistants in neurobiology-immunology

Positions available for immunologist-cell biologists to join research group studying effects of testosterone on immune system and on neuronal migration. Positions funded by N.I.H. and suitable for obtaining Ph.D. Salary within £8,675-£11,680 p.a. on Research and Analogous Staff 1B scale. Posts available for up to 3 years.

**Applications, including C.V., should be sent to Dr. Peter Behan, University Department of Neurology, Southern General Hospital, Glasgow, G51 4TF.**

(8558)A

## UNIVERSITY OF BRISTOL Electron Microprobe Operator in Microanalysis of Geological Materials

An Electron Microprobe Operator is required in the Department of Geology to oversee the establishment and operation of an electron microprobe laboratory. Experience in the microanalysis of silicate materials is essential. Preference will be given to individuals with knowledge and interest in electronics. Duties may also include development and maintenance of other electronic equipment.

The commencing salary will be at an appropriate point according to age, qualifications and experience on the Scale £12,150-£15,720 per annum.

The University does not issue application forms. Application should be made by letter including the names and addresses of three referees, and should be accompanied by a full curriculum vitae. Applications should be sent to arrive not later than 20 March 1989 to the **Registrar, University of Bristol, Senate House, Tyndall Avenue, Bristol BS8 1TH**, quoting reference JPB/35, from whom further particulars may be obtained.

(8563)A



## ***On The Leading Edge Of Clinical Cancer Research***

The Ontario Cancer Institute is Canada's largest centre for cancer treatment, research and education. The care, vision and professional collaboration of our people have earned us an international reputation for excellence.

We wish to appoint a **CHIEF RESEARCH OFFICER** to lead our highly-motivated research divisions.

The senior scientist who is appointed to this new position will have qualifications which include either a Ph.D, an M.D. or equivalent and accomplishments which would justify an appointment at the full professor level. We are seeking a person who has demonstrated significant skills in leading a team of senior investigators, working within a peer-reviewed, grant-supported environment.

Responsibilities will include leadership to a large group of independent scientists; collaboration with clinical departments to foster patient-related research; liaison with external bodies in government, universities and industry; and planning and priority-setting for research programs. A major challenge/opportunity will be to prepare for OCI's move to new expanded facilities within five years.

Canadian citizens will be given priority consideration. Scientists attracted by this important and challenging opportunity should submit appropriate documentation by May 1, 1989 to:

**Dr. D.R. Carlow, CEO and Chair of Search Committee**  
The Ontario Cancer Institute, 500 Sherbourne Street  
Toronto, Ontario M4X 1K9  
Telephone: (416) 926-4671 Fax: (416) 926-6545

**THE ONTARIO  
CANCER INSTITUTE**

(NW3395)A

## **RESEARCH SCIENTIST MOLECULAR BIOLOGIST: MOLECULAR GENETICIST**

A six-year tenure track position is available in the ICRF Laboratory of Molecular Pharmacology in Edinburgh. The overall aims of the group are to understand the factors which determine individual susceptibility to cancer as well as molecular mechanisms of tumour cell resistance to cytotoxic drugs. Candidates should have a strong background in eukaryotic or prokaryotic molecular biology and/or muscular genetics and will be expected to work independently on themes complementary to the main aims of the group.

The ICRF group is situated in the University of Edinburgh Department of Biochemistry and works closely together with other Departments in the University. The successful candidate will be considered for an honorary position within the University.

Salary range: £14,500-£23,000

For further information contact Dr. C. R. Wolf, I.C.R.F. Lab of Molecular Pharmacology, Hugh Robson Building, George Sq., Edinburgh EH8 9XD, U.K. Tel: 031-668-3343.

**Applications should be made by sending a full curriculum vitae together with the names and addresses of three referees to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX quoting reference 78/R.**

Closing date for applications: 21st April 1989

Smoking is actively discouraged.

(8609)A

**I M P E R I A L  
CANCER RESEARCH FUND**

## **RESEARCH ASSOCIATES**

**COR Therapeutics, Inc.** is a recently established and rapidly growing biotechnology company located in the San Francisco Bay Area. We specialize in the development of novel therapeutics for cardiovascular disease. We are actively recruiting highly qualified research associates to join our Research and Development Departments.

Research Associates are sought in Molecular Biology, Protein Biochemistry, Cell Biology, and Monoclonal Antibody Production.

These positions offer the opportunity to study basic mechanisms of receptor ligand interactions and to use this information to design therapeutic agents effective in the treatment of several types of cardiovascular disease. Preference will be given to individuals with prior research and/or laboratory experience.

**COR Therapeutics, Inc.** offers competitive salaries, benefits and attractive equity positions to its employees combined with the challenge and opportunity to make a significant contribution to this new organization. To apply, please send *curriculum vitae* to COR Therapeutics, Inc., 256 E. Grand Avenue, Suite 80, South San Francisco, CA 94080, ATTN: Human Resources. EOE

(NW3406)A

**COR Therapeutics, Inc.**



**UNIVERSITY OF CAMBRIDGE  
DEPARTMENT OF PATHOLOGY  
RESEARCH ASSISTANT/  
ASSOCIATE**

Applications are invited from post-doctoral scientists with experience in human cellular immunology. The position, which is available immediately and is funded for 2 1/2 years in the first instance, will involve studies on the regulation of immune responses in patients with schistosomiasis. Part of the work will be carried out in a large, multidisciplinary group in Cambridge: the successful applicant will also be expected to spend 3 to 4 months each year working in Kenya.

Salary up to £14,500, depending on age and experience plus travel and per diem expenses. Applications, with curricula vitae and the names of two referees, should be sent to: **The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP.** For informal enquiries, please contact Dr. A.E. Butterworth at the same address (telephone 0223-353492) after 20th March. (8539)A

## **Research Scientist**

### **London Medical Hospital Medical College**

Applications are invited from cell/molecular biologists for a 6-year position within the ICRF Skin Cancer Laboratory (Head: Dr Irene Leigh) at the London Hospital Medical College. The Current interests of the group are in keratinocyte differentiation *in vivo* and *in vitro*, and melanoma. The person appointed would be expected to develop a research programme with complementary interests.

The group has close links with the mucocutaneous biology group, other cancer research groups at LHMC and the epithelial biology laboratories at ICRF.

Salary range: £17,000-£25,000.

For further details contact Dr. I. Leigh, London Hospital, London E1 (01-377-7749) or Dr. M. Swain, ICRF (01-242-0200).

**Applications comprising a full cv and names and addresses of three referees should be sent to: The Recruitment Officer, Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX quoting reference 89/R.**

Closing date for applications: 5th May 1989.

(8617)A

## **I M P E R I A L CANCER RESEARCH FUND**

### **MEDICAL RESEARCH COUNCIL TOXICOLOGY UNIT**

#### **MOLECULAR OR CELL BIOLOGIST**

Applications are invited for an MRC/ICI funded post-doctoral short-term non-clinical scientific post tenable in this unit for two years (with the possibility of extension to three years).

The project involves the separation of preneoplastic cells induced by genotoxic and neo-genotoxic carcinogens using flow cytometry and elutriation and probing the separated cells for altered phenotype including oncogene expression.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information may be obtained from **The Administrative Officer, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF (Tel. 01-643 8000)** to whom applications, including a full CV and the names of two professional referees, should be sent by **23 March 1989.**

The Council is an equal opportunity employer.

(8602)A

**MRC**  
Medical Research Council

### **University College of Wales, Aberystwyth** Department of Biological Sciences

**PHYSICISTS  
BIOCHEMISTS  
BIOPHYSICISTS  
PHYSICAL CHEMISTS  
COMPUTER SCIENTISTS  
APPLIED MATHEMATICIANS**

## **SENIOR POSTDOCTORAL RESEARCHER**

A position is available under the Wolfson Research Awards Scheme to work on the development of 2-dimensional dielectric spectroscopy. The successful candidate would have an interest and experience in interdisciplinary problems and in the programming and use of Personal Computers. In the first instance, the project would involve the construction and adaptation of appropriate software for obtaining and manipulating 2-D dielectric spectra. This would be the world's first such system. Subsequently the system would be used for the characterization of a variety of specific proteins and microorganisms, and the use of such a system as a generic biosensor (see Kell, D. B. *in* Biosensors — fundamentals and applications, ed. A. P. F. Turner, I. Karube & G. S. Wilson, OUP, 1987, pp. 427-468).

This post is available for 3 years at a point up to SCP8 (£13,870 p.a.) on the PDRA 1A scale (£9,865-15,720 p.a.). There is no specific closing date for this post, but suitably qualified candidates are urged to make their interest and availability known as soon as possible.

Application forms and further particulars from the **Staffing Officer, The University College of Wales, Old College, King Street, Aberystwyth SY23 2AX (Tel 0970 623177 Ext 207) — fax 0970 611446.** Informal inquiries in confidence to Dr D B Kell on 0970 623111 Ext 3055, 0970 617172 (fax) or via BITNET (DBK@UK.AC.ABERYSTWYTH.) (8572)A

### **DIRECTOR MOLECULAR GENETICS DIVISION**

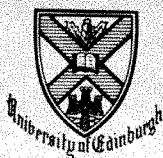
Children's Hospital National Medical Center of Washington, D.C. is seeking an individual to direct a newly created Molecular Genetics Division in the Department of Clinical Genetics. Candidates must have an M.D., Ph.D or M.D./Ph.D. degrees and have previously demonstrated skill in the field of molecular genetics. The ability to supervise a rapidly growing research team and to conduct appropriate independent research is required.

Excellent salary and benefits package with tenured appointment at the Associate Professor or Professor level in the Department of Child Health and Development of George Washington University. Position available July 1, 1989. Send curriculum vitae and brief statement of research interests to:

**Kenneth N. Rosenbaum, M.D.**  
**Director, Clinical Genetics and Genetics Laboratory**  
**CHILDREN'S HOSPITAL**  
**NATIONAL MEDICAL CENTER**  
**111 Michigan Ave., N.W.**  
**Washington, DC 20010**

Equal Opportunity Employer

(NW3392)A



University of Edinburgh and  
Agricultural and Food Research  
Council Interdisciplinary Research  
Centre For Animal Genome  
Research

## DIRECTOR

Applications are invited for the post of Director of the Interdisciplinary Research Centre for Animal Genome Research. The Centre will be established by the University and the Research Council (AFRC) during 1989 and will be supported for an initial period of 10 years (subject to review after 4 and 8 years) by a substantial direct grant from the Council. Scientific review procedures and annual reporting will conform to AFRC norms.

The Centre will pursue a research programme in the field of genetic manipulation of vertebrate animals, including the production of transgenic animals by pronuclear microinjection, germline manipulation using embryonal stem cell techniques and genetic engineering of somatic cells by stemline repopulation, and it will also act as a focus for multidisciplinary interactive projects in mammalian biology, agriculture and medicine. It will have a core of scientific, technical and managerial staff. Designated academic staff on partial secondment from the University and elsewhere will assist and advise, and will carry out agreed programmes of research. It will accommodate visiting workers from University Departments, Research Councils and Charitable Institutes and Industry on a National and International basis, by agreement with the Director.

The Director will be a distinguished research worker in a field relevant to the programme of the Centre, and preferably will have a proven experience of management. He will be responsible for the efficient and cost-effective management of the Centre; recruitment and management of staff; scientific leadership and execution of an innovative research programme; promoting links with industrial, academic and other partners; obtaining additional financial support; dissemination of results and technology transfer. He will be employed by the University and appointed in consultation with the AFRC.

The salary will be within the professorial range. Further particulars may be obtained from the **Secretary to the University, University of Edinburgh, 63 South Bridge, Edinburgh, EH1 1LS, Scotland**, to whom applications (15 copies) including curriculum vitae and the names and addresses of three referees, should be lodged not later than 15th April 1989. Overseas candidates need submit only one copy of the application. Please quote reference no. 61/89.

(8549)A

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# UNSW

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SYDNEY, AUSTRALIA



Equality of employment opportunity is University policy.

DEPARTMENT OF BIOTECHNOLOGY

## RESEARCH ASSOCIATE (Fermentation Technology)

(Ref. 108)

Applications are invited from persons holding a PhD in Chemical Engineering or a related discipline for a 3 year post-doctoral position in the Department of Biotechnology. The appointee will be an ARC Research Associate on a project involving the design, operation and analysis of bench-scale fermentors for plant tissue and organ culture. Endowed with a high level of motivation, the successful applicant will be capable of working independently and skilled at finding innovative solutions to technical problems.

Experience with fermentation equipment and cell culture is required. A background which includes plant tissue culture studies would be advantageous, but is not essential. Commencing salary in the range \$A26,617, with possible progression to \$A30,360. Generous leave and study leave are also provided.

The position is full-time and available immediately.

Further information from Dr P. Doran on (02) 697 2086.

(W5933)A

Please submit written application, QUOTING REFERENCE NUMBER and including a complete resume and the names and addresses of three referees to: The Recruitment Officer, General Staff Office, P.O. Box 1, Kensington, N.S.W. 2033, Australia, by 17 March, 1989.

## ASSISTANT PROFESSOR OF TROPICAL PUBLIC HEALTH

The Department of Tropical Public Health at the Harvard School of Public Health is searching for an Assistant Professor with expertise in cellular biology and ultrastructure of parasites. The individual will interact with other members of a multidisciplinary department including immunologists, molecular biologists, vector biologists and epidemiologists, and be expected to develop a strong research and teaching program. Experience in immunology and/or molecular biology also preferred.

Applicants should send curriculum vitae including list of publications and reprints of representative recent publications to

**ad hoc Committee on Cellular Biology  
Harvard School of Public Health  
Department of Tropical Public Health  
665 Huntington Avenue  
Boston, MA 02115**

*The Harvard School of Public Health is an equal opportunity employer and applications from women and minority groups are encouraged.*

(NW3384)A

UNIVERSITY OF SURREY  
DEPARTMENT OF CHEMISTRY

## LECTURESHIPS IN CHEMISTRY

Applications are invited for one, or possibly two, lectureship appointments in the fields of organic and inorganic chemistry, tenable from 1 September 1989. Interest in teaching computer-aided chemistry would be welcome.

Salary will be in the Lecturer range £9,260-£19,310 per annum, according to age, qualifications and experience. Superannuation under USS conditions.

Further particulars are available from the **Academic Registrar (AA), University of Surrey, Guildford, Surrey GU2 5XH, or telephone Guildford (0483) 509279**. For informal enquiries contact the Head of Department, Professor J M Pratt (0483) 509164.

Applications in the form of a curriculum vitae (2 copies), including the names and addresses of three referees, should be sent to the same address by 20th March 1989, quoting the reference 812/No.

(8560)A



# RESEARCH SCIENTIST

## RESEARCH ASSAY DEVELOPMENT

### SLOUGH

Celltech is one of Europe's foremost biotechnology companies; based at new purpose-built facilities in Slough, the company is already recognised as a world-leader in the bulk production of monoclonal antibodies and recombinant proteins for diagnostic and therapeutic use.

We are now looking for an experienced research scientist to join a group responsible for the design and development of antibody-based assays for contract research projects.

Qualified to PhD level, you must have up to three years' post-doctoral experience and a demonstrable interest in immunoassay development with enzyme-generated, fluorescent or electrochemical signal methods. You must have proven ability in the design and development of new assays. A knowledge of homogenous assays, automated assay systems, data analysis software

or monoclonal antibody derivation would be an advantage.

Working closely with project managers and commercial managers, you will also need highly developed analytical and communication skills.

Promotion prospects are excellent: Celltech values the contribution of research scientists and career development paths can take you to senior positions in scientific as well as managerial grades.

A highly competitive salary will be supported by a comprehensive benefits package which includes a pension scheme, free life assurance, discounted BUPA and relocation assistance if appropriate.

Please write with full details of your qualifications and experience to date to Mrs Jane Smith, BSc C BIOL MI BIOL, at Celltech Limited, 216 Bath Road, Slough, Berkshire SL1 4EN. Please quote Ref:173/C.



**CELLTECH**

(8616)A

## Medical Affairs Officer

**T CELL SCIENCES, INC.**, is a health care company engaged in the development and marketing of therapeutic and diagnostic products. Positions at T CELL SCIENCES include a career-enhancing environment and offer the opportunity to interface with internationally recognized scientists in converting their discoveries into products.

We are seeking an experienced Medical Affairs Officer to participate in planning, conducting and evaluating a clinical trial program involving recombinant product(s) in potential therapies for myocardial injury and events involving tissue damages. Clinical trials for future products will include autoimmune diseases and certain cancers. The individual will be responsible for identifying and coordinating multicenter clinical trials; coordinating the development of clinical protocols; reviewing and interpreting all safety and efficacy issues of the therapeutic agents under evaluation; and preparing clinical study reports, IND updates and NDA submissions.

The successful candidate will have an M.D. degree (Board Certified in Internal Medicine) with additional training in one or more of the following disciplines: immunology, cardiology, infectious diseases and rheumatology; 3 or more years of pharmaceutical drug trial experience; knowledge of regulatory affairs; and exceptional organizational, communications and management skills.

Interested candidates please forward resume/C.V., salary requirements and description of career goals/publications or abstracts to Paula R. Freeman, T Cell Sciences, Inc., 38 Sidney St. Cambridge, MA 02139.

(NW3394)A

**T CELL SCIENCES**

# SCIENTISTS

## CARDIOVASCULAR RESEARCH

**COR Therapeutics, Inc.** is a recently established and rapidly growing biotechnology company located in the San Francisco Bay Area. We specialize in the development of novel therapeutics for cardiovascular disease. We are actively recruiting a few highly qualified scientists with proven track records to join our Research Department. These scientists will join a research effort directed at the study of molecular mechanisms underlying various aspects of cardiovascular disease. The primary focus will be on growth factors and growth factor receptors, and receptors mediating vascular cell interactions.

### Molecular Biology

Expression of native, chimeric and mutant proteins. Experience with mammalian cell expression preferred. Objectives will be to use a molecular approach to understand protein function and to develop the use of expressed proteins as novel pharmaceutical agents.

**COR Therapeutics, Inc.** offers competitive salaries, benefits, and attractive equity positions to its employees combined with the challenge and opportunity to make a significant contribution to this new organization. To apply, please send *curriculum vitae* to COR Therapeutics, Inc., 256 E. Grand Avenue, Suite 80, South San Francisco, CA 94080, ATTN: Human Resources. EOE.

(NW3405)A

### Protein Biochemistry

Identification, isolation and characterization of structural and functional domains of ligands and receptors. Objectives will be to characterize structure-function relationships of these proteins and to use this information to develop receptor antagonists.

**COR Therapeutics, Inc.**

## ASSISTANT PROFESSOR OF TROPICAL PUBLIC HEALTH

The Department of Tropical Public Health at the Harvard School of Public Health is searching for an Assistant Professor with expertise in molecular biology, biochemistry or physiology or arthropods of medical importance. The candidate should have a record of productivity on the study of insects. The individual will interact with other members of a multi-disciplinary department including immunologists, molecular biologists, vector biologists and epidemiologists, and be expected to develop a strong research and teaching program.

Applicants should send curriculum vitae including list of publications and reprints of representative recent publications to:

**ad hoc Committee on Medical Entomology  
Harvard School of Public Health  
Department of Tropical Public Health  
665 Huntington Avenue  
Boston, MA 02115**

*The Harvard School of Public Health is an equal opportunity employer and applications from women and minority groups are encouraged.* (NW3385)A

## Research Scientist

Laboratory Manager of Mass Spectrometry Facility in the Clinical Research Center. 50 per cent — supervise operation of HP 5988 and HP 5970 GC/MS and Finnigan Delta E IR/MS in support of human tracer studies of substrate metabolism; 50 per cent — independent research. Requires Ph.D. in Analytical Chemistry, 2-3 years' postdoctoral experience, and direct experience in quantitative mass spectrometry and laboratory information systems management. This is a temporary appointment with possible permanent employment.

Please send two copies of both cover letter and resume referencing Job No. R88-272 to: Dr Robert Hoerr, c/o MIT Personnel Office, 400 Main Street, Bldg. E19-239, Cambridge, MA 02139.

**MIT is a non-smoking environment. MIT is an Equal Opportunity/Affirmative Action Employer.** (NW3404)A

## BIOLOGY EDITOR

The Guilford Press, a leading New York-based publisher in the behavioral sciences, is seeking a well-informed, enthusiastic, intellectually inquisitive person to develop a new program of books and journals in biology. The program will include molecular cell biology, genetics, biochemistry, neuroscience, immunology, pharmacology, and related domains.

This position provides an opportunity to work actively with prominent scholars. It is an ideal alternative for one wishing to leave academia, yet utilize his/her valuable training to advance the scientific enterprise. A graduate degree and some teaching experience are essential, unless the candidate has a strong publishing background in this field. Good writing and editing skills are a plus. The position entails extensive travel and attendance at major U.S. conferences.

To apply, please send resume and a covering letter (with salary requirements) to **Carolyn Graham, Guilford Publications, 72 Spring Street, New York, New York 10012.** (NW3399)A



**Department of Physiology  
UNIVERSITY COLLEGE LONDON**



## Appointment of three Lecturers

The vacancies arise as a consequence of appointments of members of staff to chairs elsewhere. The Department is responsible for teaching physiology to Medical and Science students. Our main research areas are Cell Physiology, Neurophysiology, Muscle (cardiac, smooth and skeletal) and Clinical Physiology. Applications are invited from active young research workers in these and in complementary fields of modern physiology. For one of the posts, we are seeking a scientist in the field of cellular and molecular aspects of endocrinology. Applications in the form of a curriculum vitae including research plans and the names and addresses of 2 referees should be sent to Professor R. Woledge, from whom further information can be obtained (01-380 7133), Department of Physiology, University College London, Gower Street, London WC1E 6BT by the 10th April 1989. Equal Opportunities Employer. (8469)A

## University of Reading

**Neuropsychopharmacology Lab.  
Department of Psychology**

## Postdoctoral Research Fellow and Postgraduate Research Assistant

required for a project focusing on drugs of abuse and the interaction between dopamine and opioid peptides. The FELLOW will use extracellular single unit recording and microiontophoretic techniques. Candidates should have a PhD in a neuroscience field; experience with electrophysiological techniques is not essential. The appointment is for 3 years; salary range £9,865-£11,070 p.a. The OFFICER will be involved in behavioural work relevant to the electrophysiological studies. The appointment is for 19 months at a salary of £8,675-£9,260 p.a. Applicants should possess a relevant first degree. Closing date for both positions is 30 March 1989. Informal enquiries to Dr David Clark on (0734) 318532. **Apply for Application Form** and further particulars to the Personnel Officer, University of Reading, Whiteknights House, P.O. Box 217, Reading, RG6 2AH, telephone (0734) 318754. Please quote ref. R8912. (8578)A

**COLEG PRIFYSGOL  
COGLEDD CYMRU  
UNIVERSITY COLLEGE  
OF NORTH WALES**

DEPARTMENT OF CHEMISTRY

## LECTURESHIP IN ORGANIC CHEMISTRY

Applications are invited for a Lectureship in Organic Chemistry in relation to the UGC-approved expansion of the Department. The successful applicant is likely to have had postdoctoral experience but no designation of the field of research is being made.

The appointment will be to the Universities' Lecturer Scale Grade A (£9,260-£14,500 p.a.) and will commence on 1 October 1989 or such other date as may be arranged.

Applications (two copies) giving full details of age, qualifications and experience together with the names and addresses of three referees should be sent by Friday, 30th April, 1989, to **Mrs M E MacDonald, Assistant Registrar, University College of North Wales, Bangor, LL572DG** from whom further particulars may be obtained. Informal enquiries may be made to the Head of the Chemistry Department, Professor C J M Stirling, FRS (telephone: 0248 351151 ext 2375). (8604)A

**UNIVERSITY OF  
NOTTINGHAM**  
Department of  
Physiology &  
Environmental Science  
**LECTURER IN  
ANIMAL  
PHYSIOLOGY**

Applicants should possess training to post-doctoral level in animal physiology with research interests in growth, reproduction or lactation and preferably experience in molecular biology, immunology and/or endocrinology.

Salary within the range £9,260-£19,310 per annum.

Further details and application forms, returnable not later than 23 March 1989, from the **Personnel Office, University of Nottingham, University Park, Nottingham NG7 2RD (Tel 0602 484848 ext 3355). Ref No 1227.** (8556)A

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in science

**THE UNIVERSITY OF LEEDS**  
DEPARTMENT OF PURE AND  
APPLIED BIOLOGY  
**TWO LECTURESHIPS IN  
BIOLOGY**

Applications are invited for two posts of Lecturer available from 1 October 1989.

The successful candidates will have demonstrated an ability to initiate and carry out high-quality innovative research in the following fields:

1. Vertebrate Zoology, especially bio-mechanics and/or behaviour of vertebrates (ref no: 56/25).
2. Plant Molecular Biology (ref no: 56/26).

It is intended that the new appointments should consolidate existing departmental strengths through both independent and collaborative research.

Salaries will be on the scale for Lecturers Grade A (£9260-£14500), according to qualifications and experience.

Application forms and further particulars can be obtained from and completed applications forwarded to, the Registrar, The University, Leeds LS2 9JT (tel (0532) 333963 — direct line) quoting appropriate reference No. Closing date for applications 23 March 1989. (8593)A

**University of Nottingham**  
**Department of Physiology and  
Environmental Science**

Applications are invited for a Lecturer in Computer Modelling in Environmental Biology, commencing 1 September 1989. The ideal candidate will have research experience in the computer modelling of Biological systems and an interest in teaching statistics and computing to biologists.

Salary within the range £9,260 — £19,310 per annum depending upon age and experience.

Further details and application forms, returnable not later than 31 March 1989, from the **Personnel Officer, University of Nottingham, University Park, Nottingham NG7 2RD (Tel 0602 484848 ext 3355). Ref No 1225. (8555)A**

**ENVIRONMENTAL  
PATHOLOGY/TOXICOLOGY**  
NRSA-supported  
**PRE- AND POSTDOCTORAL  
POSITIONS**

available with research emphasis on cellular and molecular effects (mutagenesis, neurotoxicity, bio-transformation, DNA dosimetry) of toxic chemicals (especially trace metals, aromatic hydrocarbons, ozone respiratory epithelial injury, aflatoxins).

Inquiries: **Dr. N. K. Mottet, Program Director, Department of Pathology, SM-30, University of Washington, Seattle, WA 98195. U.S. citizen/permanent residents. An Equal Opportunity/Affirmative Action Employer. (NW3397)A**

**UNIVERSITY OF  
ZIMBABWE**

Applications are invited for the following posts:

**DEPARTMENT OF  
PHARMACY  
PROFESSORSHIP**

Applicants must have a first degree in Pharmacy (registrable in Zimbabwe) with appropriate higher degrees to the doctorate level, and extensive teaching, research and professional experience. The successful candidate will be required to undertake a major role in the continued development of undergraduate and postgraduate work in the Department and to conduct and lead appropriate research.

**ASSOCIATE  
PROFESSORSHIP/SENIOR  
LECTURESHIP/  
LECTURESHIP**

Applicants should be pharmacy graduates with appropriate post-graduate qualifications and clinical/hospital experience. The successful candidate will participate in the well developed teaching and research programmes in clinical pharmacy in the Faculty of Medicine. Pharm.D or ward pharmacist preferred.

*Salary Scales: Medicine/Veterinary (Including Professional Supplement) Lecturer: Z\$23,868-Z\$32,184; Senior Lecturer: Z\$33,132-Z\$35,172) Associate Professor: Z\$36,132-Z\$36,624; Professor: Z\$36,948 (fixed). Non Medical: Lecturer: Z\$19,860-Z\$28,176; Senior Lecturer: Z\$29,124-Z\$31,572; Associate Professor: Z\$32,532-Z\$33,624. Appointment on the above scales will be according to qualification and experience.*

**CONDITIONS OF SERVICE**

Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended. Applicants should quote reference number ASA/2/01/89 and submit six copies of applications giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees to the Deputy Registrar (Administration), University of Zimbabwe, P O Box MP 167, Mount Pleasant, Harare, Zimbabwe. Telegrams: UNIVERSITY; Telex 4-152 ZW. Applicants from outside Zimbabwe should also send a copy to the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK, from whom further details may be obtained.

Closing date for receipt of applications is 24 March 1989.

(W5935)A

**MEDICAL MOLECULAR BIOLOGY UNIT  
DEPARTMENT OF BIOCHEMISTRY  
UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL  
OF MEDICINE**

Applications are invited for a postdoctoral position in the above unit supported by Action Research for the Crippled Child. The person appointed will undertake the cloning and characterization of the gene encoding a novel neuron-specific transcription factor which binds to the octamer motif in cellular genes and related TAATGARAT elements in herpes simplex virus immediate-early genes.

Post available from May 1st or thereafter by arrangement. Experience in either neurobiology, virology or molecular biology would be an advantage but not essential as training will be provided. Salary on RA1A scale from £11,680 to £15,720 depending on age and experience plus £1650 London Allowance.

Applications with cv and names of two referees or informal enquiries to Dr D S Latchman, Medical Molecular Biology Unit, Windeyer Building, Cleveland Street, London W1P 6B. Tel: 01-380 9343 or 01-636 8333 ext 3079.

Equal Opportunities Employer

(8566)A

**ROYAL POSTGRADUATE MEDICAL SCHOOL  
(University of London)**

**DEPARTMENT OF CHEMICAL PATHOLOGY  
RESEARCH ASSISTANT**

A graduate research assistant is required to investigate the involvement of the amylin gene in type II diabetes. Studies will include the identification of polymorphisms in the amylin gene for use in linkage analysis in type II diabetic families and the development of immunoassays for analysis of amylin gene expression. Although previous experience is desirable, training can be given to a suitably qualified candidate and the opportunity exists to register for a higher degree. The project is supported by a grant of 2 years duration from The British Diabetic Association.

Starting salary is up to £9413 (inc. London weighting) according to age and experience.

Application forms and further details are available from the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN (tel: 01-740 3204) quoting ref: ACM7.**

Closing Date: 31 March 1989.

(8588)A

**UNIVERSITY OF BIRMINGHAM  
CRC — Department of Cancer Studies  
Research Fellow**

Applications invited for a post-doctoral research fellow to join a group of six working on (a) the role of specific chromosome translocations in leukaemogenesis in patients with ataxia telangiectasia (A-T), and (b) the location of the A-T gene. Experience in some recombinant DNA techniques is preferred. Those expecting to complete a PhD by October 1989 are also encouraged to apply.

Post for 3 years. Salary £9,865-£15,720 with superannuation. Maximum starting £11,680.

Informal enquiries to Dr A M R Taylor on 021-414-4471. Application forms and further particulars from **Senior Assistant Registrar, Medical School, Birmingham, B15 2TJ** to whom completed applications (3 copies) should be sent by 31st March 1989.

Quote Ref RF/CS/AMT.

ANEQUAL OPPORTUNITIES EMPLOYER.

(8569)A

**GREENADDER INDUSTRIAL  
WORLDWIDE PLACEMENTS  
FOR**

**PHARMACISTS, ANALYSTS, RESEARCH SCIENTISTS  
2A WESTBRIDGE ROAD, LONDON SW11 3PW TEL: 01-223 3497**

(8495)A



**UNIVERSITY OF LIVERPOOL  
MAGNETIC RESONANCE RESEARCH CENTRE  
SENIOR DESIGN AND MAINTENANCE OFFICER**

To design, construct, test and maintain patient safety equipment, including such items as ECG Monitors, Defibrillators, Ventilators and, in addition, to carry out other electro-mechanical development projects such as the construction of the Radiofrequency antenna (coils) for Magnetic Resonance Imaging and Spectroscopy as well as other MR related projects.

The post requires previous experience in the design and maintenance of clinical equipment. It will involve working closely with the NHS on matters relating to patient safety. Familiarity with the relevant safety regulations will be essential. Candidates should be qualified to HNC level or equivalent, although a degree would be an advantage.

Salary within the range £9,277-£11,185 per annum (under review).

Quote Ref: PER/649/N.

Closing date: Friday, 17th March 1989.

*An Equal Opportunity Employer.*

Application forms available for the **Director of Staffing Services (NAS), The University, P.O. Box 147, Liverpool L69 3BX.** (8575)A

**THE INSTITUTE OF CANCER RESEARCH  
Fulham Road, London**

**A RESEARCH OFFICER** is required to work on a project that will be run jointly by Dr. B Reeves (Section of Pathology) and Dr. C.S. Cooper (Section of Chemical Carcinogenesis). This work will involve the identification of genetic changes that may be involved in the development of human soft tissue tumours and will require the use of several molecular genetic techniques including Southern analysis and production of genomic libraries. The post would be suitable for a science graduate with a particular interest in molecular biology or for someone with experience in molecular genetic techniques.

The salary depending on qualifications and experience is in the range £8321 to £11706 p.a. inclusive.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Applications should be sent, in duplicate, with the names and addresses of two referees to the **Personal Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference number 3.89.T.N.66** (8545)A

**SYNTHETIC PEPTIDE CHEMIST**

A private, non-profit research institute is seeking a peptide chemist to head up a small unit within a department to design and synthesize biologically active peptides, analogs and fragments for physiological studies and raising of antibodies. A strong background in organic synthesis is required and experience in solid phase peptide synthesis methodology is helpful but not essential. Initial salary, equipment and supplies will be provided with the understanding that the candidate is expected to participate with a group of biologists, biochemists and molecular biologists to secure funding from NIH to study the physiology of the polypeptide regulators in ovarian follicular fluid.

If interested, please send curriculum vitae, statement of research interest and names of at least three references to **Nicholas Ling, Ph.D., Head of Department of Molecular Endocrinology, The Whittier Institute for Diabetes and Endocrinology, 9894 Genesee Avenue, La Jolla, CA 92037.**

(NW 3398)A

**UNIVERSITY OF ULM**

**Position available**

**Molecular biology –  
regulation of hemopoiesis**

We offer a position for at least 3 years as a group-leader for a scientist with good background in molecular biology. Experimental hematology and stem cell research is well represented in our institute.

The applicant has the possibility to participate in the formulation of the research program that should address itself to the mechanisms of stem cell replication and differentiation.

Applications to **Prof. Dr. T. M. Fliedner, Institute of Occupational and Social Medicine, University of Ulm, Albert Einstein Allee 11 D – 7900 Ulm.** (W5930)A

**STUDENTSHIPS**



**FELLOWSHIPS IN  
JAPANESE NATIONAL  
LABORATORIES**

The Japanese Science and Technology Agency (STA), in association with the Royal Society, is offering postdoctoral fellowships of 6 to 24 months' duration tenable in Japanese national laboratories and public research corporations, to be taken up before the end of March 1990. Applications are invited from suitably qualified scientists and engineers under the age of 45, to research in any science or engineering discipline except military R&D. Fellowships include a return air fare, a monthly stipend, family allowance where necessary and health insurance. Provision for Japanese language training will also be made, but the working language for holders will normally be English. Successful candidates will subsequently be able to apply to the Royal Society for 'return-home' fellowships tenable in the UK.

Applications must be accompanied by a letter of invitation from the host institution and a clear research proposal. The Royal Society can provide on request information on the institutes in which the fellowships are tenable and advice on making initial contact. Those interested in applying should contact:

**The Executive Secretary (Ref: KK),  
The Royal Society,  
6 Carlton House Terrace,  
London SW1Y 5AG.  
Telephone: 01-839 5561 ext 309.**

CLOSING DATE FOR APPLICATIONS: 15 JUNE 1989. (8550)E



**THE ROYAL SOCIETY  
OF EDINBURGH**

**SUPPORT RESEARCH  
FELLOWSHIPS**

Applications are invited from Lecturers, aged under 45, holding permanent appointments for at least five years, in any Scottish University, Central Institution or College of Technology.

Support Fellowships enable temporary replacements to be appointed whilst the Research Fellows take study leave for twelve months, beginning 1st October 1989, to carry out advanced research in any discipline.

Application forms and further details are available from the **Executive Secretary, The Royal Society of Edinburgh, 22-24 George Street, Edinburgh EH2 2PQ,** and must be returned by 31st March 1989. (8596)E

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## UNIVERSITY OF OXFORD

### NUFFIELD DEPARTMENT OF ANAESTHETICS and GREEN COLLEGE

#### POST DOCTORAL RESEARCH ASSISTANTSHIP (Respiratory Physiology) and

#### JUNIOR RESEARCH FELLOWSHIP, GREEN COLLEGE

Applications are invited from Physiologists with a PhD and experience in respiratory techniques, for a Wellcome Trust Post Doctoral Research Assistantship, to work with Dr C E W Hahn and Professor M K Sykes on the evaluation and development of a new technique for assessing cardio-respiratory function in the experimental laboratory and Intensive Care Unit. The project will involve microcomputer control of a system to deliver a sinusoidal inert gas forcing function to inspired air, and the subsequent analysis of the expired gases by mass spectrometry. Derived cardiopulmonary indices will be compared with those obtained by conventional techniques in the normal and sick lung.

The Applicant will be part of a team containing a medically qualified Research Fellow, a mathematician and technical support staff. A suitable applicant for this 3 year post may be offered a Junior Research Fellowship at Green College, for the grant duration. This will include membership of the Common Room, meals at Common Table and eligibility for membership of the Governing Body.

**Salary within range £9,865 – £13,870 on University Academic Related (1A) Scale.**

The post is available immediately, although the starting date is negotiable.

Informal enquiries and/or applications, including full CV and names of two referees, may be made to:

**Dr C E W Hahn, Nuffield Department of Anaesthetics, University of Oxford, Radcliffe Infirmary, OXFORD OX2 6HE. Tel: Oxford (0865) 727342 or 816779 (direct lines).**

*The University is an Equal Opportunity Employer.*

(8562)E

## ROCKEFELLER FOUNDATION BIOTECHNOLOGY CAREER FELLOWSHIPS

The Rockefeller Foundation announces a program of career development fellowships designed to enable scientists from developing countries, trained at outstanding centers for advanced research on biotechnology, to continue to work at those or other institutions for three months each year, over a period of at least three years, conducting advanced research and keeping abreast of new developments in their fields. The program will focus upon the development and application of advances in molecular and cellular biology and immunology relevant to agriculture, health, and reproductive biology.

Funding will be shared between the Foundation and the host laboratory, with the Foundation providing travel and per diem support. It is hoped that the fellowships will encourage the establishment of ongoing working relationships between outstanding younger scientists working at third world institutions, and research teams at advanced laboratories.

Applicants to this program should have at least Ph.D.- or M.D.-level training, a proven record of scientific productivity, and a permanent position at a research or teaching institution in their home country. A written project proposal must be developed and submitted jointly by the candidate and the laboratory sponsor.

Information about application procedures can be obtained by writing to **Biotechnology Career Fellowships, Fellowship Office, Rockefeller Foundation, 1133 Avenue of the Americas, New York, New York 10036, USA.**

(NW3382)E

## POSTDOCTORAL FELLOWSHIPS

The Imperial Cancer Research Fund is one of the largest independent cancer research institutes in Europe, employing approximately 400 scientists and clinicians. It has a wide ranging programme in fundamental, applied, and clinical cancer research.

### RESEARCH FELLOWSHIP IN CELL BIOLOGY

**£14,000 – £17,000**

Applications are invited for a post-doctoral fellowship, tenable for three years, to join a group working on the sorting of membranes during mitosis. These studies focus on the division of the Golgi apparatus and utilize a variety of techniques including immunoelectron microscopy, cell-free reconstitution and yeast molecular genetics. Preference will be given to applicants familiar with one or more of these techniques.

Further information can be obtained from Graham Warren, (01)-242-0200 Ext. 2281.

Closing date for applications: 28th April 1989. Ref: 79/R.

### RESEARCH FELLOWSHIPS IN GENETIC RECOMBINATION

**£13,300 – £16,000**

Applications are invited for two postdoctoral positions:

- (1) The mechanism of homologous pairing and strand exchange by the *E. coli* RecA protein
- (2) Resolution of recombination intermediates (Holliday junctions) in vitro.

Candidates for both positions should have experience in the techniques of molecular biology. Additional experience in cloning and gene manipulation would be advantageous for project (2). The fellowships are available at the ICRF's Clare Hall Laboratories situated 10 miles north of London and are tenable for a period of three years. Ref: 80/R.

**Applications, for any of the positions, comprising a full curriculum vitae and the names and addresses of three referees should be sent to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX quoting the appropriate reference. Smoking is actively discouraged** (8608)E

**I M P E R I A L  
CANCER RESEARCH FUND**

## EMBO

### EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

#### SHORT TERM FELLOWSHIPS in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from **Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G.** (W5706)E

## National Cancer Institute-Frederick Cancer Research Facility BIONETICS RESEARCH, INC.-BASIC RESEARCH PROGRAM POSTDOCTORAL FELLOWSHIPS

BRI-Basic Research Program Postdoctoral Fellowships are awarded for 1-2 years with stipends ranging from \$24,000 to \$30,000/year. The senior staff members and their research interests are

**George Vande Woude:** molecular basis of neoplastic transformation

**Stephen Hughes:** structure and function of HIV reverse transcriptase and integration protein; expression of cytoskeletal genes; retroviral vectors; transgenic birds

**George Pavlakis:** eukaryotic gene regulation; molecular mechanisms of cell transformation; molecular biology of HIV and pathogenesis of AIDS

**Peter Johnson:** mammalian transcription factors; molecular basis of tissue-specific gene expression

**Stephen Oroszlan:** immunochemistry and protein chemistry of retroviruses; structure and function of retroviral gene products; proteases

**Alan Rein:** retroviral genetics; functional analysis of retroviral genes using natural and synthetic mutants

**Nancy Rice:** molecular biology of retroviruses; oncogene expression

**William Lijinsky:** environmental carcinogenesis; mechanisms of carcinogenesis by nitrosamines and related compounds

**Anthony Dipple:** carcinogen-DNA interactions; polycyclic aromatic hydrocarbon carcinogenesis and mutagenesis

**Robert Moschel:** chemical synthesis of carcinogen-modified DNA; physical chemistry of carcinogen-DNA interactions; DNA adduct-induced mutagenesis in bacteria and mammalian cells

**Christopher Michejda:** biochemistry of carcinogen activation; DNA alkylation; chemotherapeutic agents

**Jeffrey Strathern:** cell type regulation; genome rearrangement, recombination, and DNA repair in yeast

Send a curriculum vitae and the names of three references to Dr. Maurice L. Guss, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, P.O. Box B (Rm 48), Frederick, Maryland 21701.

An equal opportunity/affirmative action employer M/F/H/V

**David Garfinkel:** molecular biology of the yeast retrotransposon Ty1; genome rearrangement and evolution

**Amar Klar:** gene regulation and homothallic mating-type switching in fission and budding yeast

**Stuart Austin:** chromosome stability in bacteria: regulation of plasmid replication and distribution of copies to daughter cells

**Richard Fishel:** molecular mechanisms and biochemistry of genetic recombination

**Donald Court:** regulation of gene expression by transcription initiation, transcription termination, and RNA processing

**Neal Copeland:** molecular genetics of murine leukemogenesis; developmental genetics; transposable elements and retroviruses

**Nancy Jenkins:** insertional mutagenesis by retroviral DNAs; transgenic mice; molecular biology of mouse development

**Luis Parada:** use of pluripotential stem cells to study molecular biology of development and the role of oncogenes; *in situ* hybridizations

**Peter Donovan:** development of the germ line; germ line mutations; cell matrix interactions

**Alexander Wlodawer:** structure of enzymes and oncogene products studied by X-ray diffraction

**Irene Weber:** crystallographic investigation of proteins, DNA, and protein-nucleic acid interactions

**J. Ronald Rubin:** crystallographic investigations of ligand-macromolecule interactions and drug-nucleic acid interactions

(NW3340)E

## CALIFORNIA INSTITUTE OF TECHNOLOGY THE MYRON A. BANTRELL POSTDOCTORATE FELLOWSHIP FOR SCIENTIFIC RESEARCH IN GEOLOGICAL AND PLANETARY RESEARCH

A fellowship will be awarded for postdoctoral studies in geological and planetary sciences at the California Institute of Technology. This fellowship carries an annual stipend of \$32,000 from Fall 1989, and in addition offers a research-expense fund of \$1,000 per year. The duration of the fellowship will normally be for 1 year. Applicants must be US citizens.

This fellowship program has been established to offer scientists, typically within two years of receipt of the Ph.D., the best possible opportunity to develop their talents. It is the intent of this program to identify and support innovative and creative work in the earth and planetary sciences, with particular emphasis on interdisciplinary work. In addition to individuals with degrees in geology, geophysics, geochemistry, or planetary science, we urge highly talented individuals with training in chemistry, physics, biology or computer sciences to apply. The Caltech faculty is currently active in Planetary Science, Geology, Geophysics, Geochemistry and Cosmochemistry.

Application forms may be obtained from **Professor G. J. Wasserburg, Chairman, Division of Geological and Planetary Sciences, 170-25, California Institute of Technology, Pasadena, California 91125.**

COMPLETED APPLICATIONS WITH REFERENCES  
SHOULD ARRIVE AT CALTECH  
BEFORE MAY 1, 1989.

Fellowship candidates will automatically be considered for other available post-doctoral positions at Caltech in their fields of interest.

*Caltech is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.*

(NW3377)E

## RESEARCH DEVELOPMENT AWARDS AT THE NATIONAL ZOOLOGICAL PARK

The National Zoological Park (NZP) is offering **Research Fellowships** for scientists to complete a major piece of work in their specialty, and to gain experience in research opportunities specific to zoological parks particularly in the areas of evolutionary biology, animal behavior, physiological zoology, ecology, sociobiology, reproductive physiology, conservation biology, and veterinary sciences. Applicants with a distinguished record of research contributions and those whose interests complement the NZP's current professional research staff are preferred.

Each award is for a maximum of 3 years; stipends to be commensurate with professional level and experience. Applicants must be at least 3 years post-doctorate. Timing and duration of the Fellowships are flexible, but the appointment must be activated within a year of the award and may not exceed a total of 36 months in duration (awards may be divided into several intervals, not less than 6 months each).

Field research is appropriate, however. Fellows are to be primarily based at the NZP to maximize interactions and collaborations with NZP staff. Individuals who have received support from or through the NZP for a significant period during the preceding 5 years are ineligible to apply.

For information contact: **Dr. Devra G. Kleiman, Assistant Director for Research, National Zoological Park, Washington, DC 20008.** Application deadline is 1 July 1989.

(NW3391)E



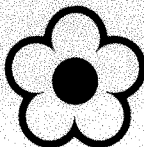


## Royal Postgraduate Medical School

(University of London)

### MRC/LRF LEUKAEMIA UNIT

# MRC



## Department of Haematology

One or two Research Fellowships to work in the above Unit have been made available through the generosity of the Hunting Gate organisation. The Unit has research programmes in the molecular and cell biology of leukaemia, and its primary objective is to improve the management of patients with leukaemia. Applications are invited from young investigators either medically qualified or with a Ph.D. who are interested in work on the molecular basis of leukaemia, and who have relevant research experience.

Additional details can be obtained and an informal meeting can be arranged with the Director Prof L. Luzzatto and members of the Unit by phoning 01-740 3234.

The appointment is for three years with salary on the University scale.

Applications, including cv, bibliography, statement of research interests and intentions and names of three scientific referees to the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN** quoting ref: **HG/LRF.** (8586)E

## Postdoctoral Fellow Program



### Gene Expression in Plants

Applications are invited for Postdoctoral Fellowships to join a dynamic and ongoing basic research

program in plant molecular biology. Project includes the characterization of genes expressed in specific cells and tissues, and the study of promoter function.

Highly motivated individuals with a strong background in molecular biology should apply by sending a CV and the name of three references to: Calgene Fellow Program, 1920 Fifth Street, Davis, CA 95616. An equal opportunity employer.



# CALGENE

(NW3388)E

## THE MORRISON MEMORIAL POSTDOCTORAL FELLOWSHIP

The Department of Biochemistry, St. Jude Children's Research Hospital, is enlarging its research efforts in the area of the molecular and cellular biology of signal transduction. As part of this expansion, the Board of Governors has established a memorial fellowship in honor of Dr. Martin Morrison, the founding chairman of the Department. The research programs within the Department are well equipped and offer recent Ph.D. or M.D. graduates the opportunity to participate in energetic, multidisciplinary groups dealing with a range of basic and clinically oriented research. Current research programs within the Department include:

- W.Y. Cheung: Regulatory properties and biological functions of calmodulin;  $Ca^{2+}$  regulated enzymes.
- J. Cleveland: Growth factor regulation of gene transcription.
- V.A. Fried: The ubiquitin system and post-translational regulatory pathways.
- J.N. Ihle: Mechanisms of hematopoietic stem cell growth regulation, differentiation and transformation.
- S. Jackowski: Regulation of phospholipid metabolism and the production of lipid-derived second messengers.
- C. Rock: Hormone and growth factor stimulated phospholipase C.
- E. Thomas: Antimicrobial and antitumor mechanisms of leukocytes.

Applicants should submit a brief statement of their research interests, curriculum vitae, reprints and names of three references to:

**The Morrison Postdoctoral Fellowship  
Department of Biochemistry  
St. Jude Children's Research Hospital  
332 N. Lauderdale  
Memphis, TN 38101**

*An Equal Opportunity/Affirmative Action Employer*  
(NW3401)E

### INTERNATIONAL CANCER RESEARCH FELLOWSHIP

The Hayashibara Mutual Aid Fund, a non-profit making organization within the Hayashibara Group announces a fellowship program. Two awards will be made annually. Fellowship will be taken up at the newly opened Fujisaki Cell Center which is devoted for the basic and applied research related to the problems in human cancer. The fellowship is normally made for one year and is renewable for up to five years. The fellowship will be expected to commence within six months of the announcement of the awards. The awards are for high quality research work in one of the three categories described below.

1. Fundamental Leukemia-Lymphoma Research,
2. Cytokine-Lymphokine Research,
3. Hematopoietic Cell Cultures.

Qualified person who has a Ph.D., an M.D. or equivalent qualifications will be awarded on merit to suitably qualified research proposal and experiences. Additional information and application forms will be obtained from: **Jun Minowada, M.D., Director, Fujisaki Cell Center, 675-1, Fujisaki, Okayama 702, Japan.** (W5829)E

### Hebrew University Medical School

## POSTDOCTORAL FELLOWSHIPS

Available to study gene regulation and DNA replication in eukaryotes and prokaryotes in the laboratories of Howard Cedar, Aharon Razin and Gad Glaser. A background in Molecular Biology is required. Funding for 2-3 years.

Send C.V. and names of three references to **Dr. Howard Cedar, Dept. of Cellular Biochemistry, Hebrew University Medical School, P.O.B. 1172, Jerusalem. ISRAEL 91010.** (W5920)E

## PHARMACEUTICAL SCIENCES INSTITUTE RESEARCH COUNCIL STUDENTSHIPS AVAILABLE IN OCTOBER 1989

The Institute is actively involved in a broad drug research programme ranging from eukaryotic and prokaryotic cell biochemistry and biology, molecular pharmacology and toxicology, synthetic organic chemistry, X-ray crystallography and computer graphics to drug formulation, pharmacokinetics and clinical trials of drugs emanating from the Institute.

Research takes place in SIX research groups. The presence of 16 postdoctoral fellows, working in new well-equipped laboratories, facilitates an excellent environment for research training. Elements of formal training exist in some groups.

The present postgraduate population of 50 is divided between the following groups:

**Toxicology Group:** molecular toxicology of formamide and nitroalkane solvents; cellular ionic homeostasis; mechanisms of toxin-induced cell death.

(Group Convenor: Dr. Andreas Gescher)

**Microbiology Group:** influence of growth environment upon microbial pathogenicity and antibiotic sensitivity; chemical and immunochemical composition of the microbial surface.

(Group Convenor: Professor Michael Brown)

**Drug Development Group:** the design and synthesis of new drug entities for the treatment of AIDS; pro-drugs and soft-drugs; X-ray crystallography, computer graphics; topical delivery, drug encapsulation and controlled release formulations; drug targeting.

(Group Convenor: Professor Malcolm Stevens)

**Drug Mechanisms Group:** physiological and pharmacological determinants of vascular reactivity; receptor mechanisms; calcium homeostasis; ischaemic conditions; pharmacological mechanisms of anxiety and migraine.

(Group Convenor: Professor Brian Ferry)

**Cancer Research Campaign Experimental Chemotherapy Group:** colon and leukaemic tumour cell differentiation; the activation of stress genes in cell differentiation and death; tumour-host metabolism and cancer cachexia; membrane biochemistry and pharmacology, ion flux in mitogenesis; cellular and biochemical pharmacology of phorbol esters; synthesis of DNA-sequence specific agents, inhibitors of tyrosine protein kinases; drug metabolism of azido-containing drugs and imidazotetrazinones.

(Group Convenor: Dr. John Hickman)

**Pharmacy Practice Group:** Drug utilisation studies; research on the advisory role of the pharmacist; applications of information technology in pharmacy practice.

(Group Convenor: Mr. Michael Jepson)

At least TWELVE research training studentships will be available in October 1989. Candidates should have, or expect to obtain, a first or upper second class honours degree in pharmacy, the life or physical sciences.

For further details please write to the appropriate group convenor (see above), Pharmaceutical Sciences Institute, Aston University,



Birmingham B4 7ET, enclosing a full CV and the names of two referees.  
Closing date: 1st April, 1989.

(8610)F

### ASTON UNIVERSITY

#### UNIVERSITY OF READING

##### School of Animal and Microbial Sciences Department of Biochemistry & Physiology POSTGRADUATE STUDENTSHIP

Applications are invited for a postgraduate research studentship to study the control of gene expression in muscle in relation to animal growth. The successful applicant will join the Department's active Growth Biochemistry and Molecular Biology Groups and will receive an excellent training in the techniques of molecular biology as applied to animal science. The studentship is sponsored by the Ministry of Agriculture, Fisheries and Food.

Applicants should have or expect to obtain at least an upper second class degree in a relevant science subject. Applications, including a full curriculum vitae and the name and addresses of two academic referees should be sent to **Dr M. A. Lomax or Dr D. Savva, Department of Biochemistry & Physiology, University of Reading, Whiteknights, PO BOX 228, Reading RG6 2AJ (Tel. 0734 875123).** (8540)F

#### THE UNIVERSITY OF LEEDS

##### DEPARTMENT OF BIOCHEMISTRY STUDENTSHIPS IN PLANT BIOTECHNOLOGY

Two SERC studentships are available that have been 'ear-marked' for research projects in the laboratory of Dr. Dianna Bowles.

- (i) plant molecular biology: isolation and characterization of genes encoding proteins involved in cell signalling;
- (ii) plant molecular pathology: isolation and characterization of defence-related genes identified in resistance responses to nematodes.

Both projects will be carried out within a multi-disciplinary team combining expertise of cell biology, biochemistry and recombinant DNA techniques.

Applicants should have, or hope to gain shortly, a good first degree in Biochemistry, Molecular Biology or Plant Molecular Sciences.

Informal enquiries to **Dianna Bowles (0532-333125)** and written applications to the **Departmental Secretary, Department of Biochemistry, University of Leeds, LS2 9TJ.** (8592)F

## 1989 PHD OPPORTUNITIES

Celltech Limited is one of Europe's leading biotechnology companies. We operate from our excellent purpose-built facilities in Slough, and are among the leaders in the research and manufacture of protein-based drug products. We now invite applications for two:

### 3 YEAR RESEARCH STUDENTSHIPS for October 1989 in the following fields

- RO1) Isolation and analysis of abzymes using microbial secretion systems.**
- RO2) Culture of mammalian cell lines producing recombinant proteins.**
- RO3) Expression and characterisation of human immunodeficiency virus antigens.**
- RO4) Pharmacological control of inflammation using monoclonal antibodies to adhesion molecules.**
- RO5) Investigation of the reaction mechanisms of metalloproteinases, relevant to human connective tissue disorders.**

Applicants should have or expect to attain a First or Upper Second Class Honours degree in a relevant subject.

Please send a C.V. including the name of two referees, and indicate the research project(s) reference in which you are interested to: Jane Smith, Celltech Limited, 216 Bath Road, Slough, Berks. SL1 4EN. Closing date: 31.03.89 (8615)F



# CELLTECH

ASSISTANTSHIPS

### UNIVERSITY OF OXFORD Department of Experimental psychology

#### RESEARCH ASSISTANTSHIP(S) IN THE NEUROPHYSIOLOGY OF TASTE, VISION OR MEMORY

Applications are invited for post-doctoral or graduate positions to analyse the activity of single neurons in cortical taste and visual areas and limbic structures such as the hippocampus in the primate. Applications will be particularly welcome from scientists with interests in neurophysiology, information processing in the cerebral cortex, and/or mechanisms of learning and memory. The salaries are on the RS1A scale, £9,865-£15,720, or the RS1B scale £8,675-£13,365. Applications including the names of two referees, or enquiries, to Dr. E.T. Rolls, University of Oxford, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD. The University is an Equal Opportunities Employer (8579)P

### UNIVERSITY OF DUNDEE DEPARTMENT OF BIOLOGICAL SCIENCES POSTDOCTORAL ASSISTANT CYANOBACTERIAL TOXINS

Applications are invited from post-doctoral biologists, microbiologists, botanists and biochemists for a research assistantship to study cyanobacterial toxins, their persistence and removal from freshwaters. The appointee will join an established group studying the production, properties and actions of peptide and alkaloid toxins of the major freshwater bloom-forming cyanobacteria. The position is for TWO YEARS and is available immediately. Salary will be on the PDRA scale (£9,865 - £15,720), commensurate with age and experience. Applications with CV (2 copies) including the names and addresses of 2 referees to the Personnel Office, The University of Dundee, DD1 4HN, UK quoting ref. EST/405/89/N. Further information may be obtained by telephoning (0382) 23181 Ext. 4767. (8543)P

## BRITISH DIABETIC ASSOCIATION

### RESEARCH STUDENTSHIPS

Applications are invited for three studentships recently awarded by this Association. Applicants should be science graduates who possess, or expect to obtain a 1st class or Upper Second class honours degree. The studentships will be of three years duration and should lead to the successful candidate achieving a PhD. The annual maintenance grant will be £4500. All three studentships are available from 1st October 1989 and applications should be made to the respective Universities as detailed below by 3rd April 1989, enclosing a c.v. and the names of two academic referees.

1. UNIVERSITY OF BRISTOL. "Action of insulin on mRNA translation"  
This project will investigate the molecular mechanisms by which insulin stimulates protein synthesis. The successful applicant will receive training in a variety of techniques including the use of animal models, protein isolation, the assay of protein synthesis and the activity of translational components and protein and peptide analysis.  
Applications should be sent to Dr C Proud, Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, U.K. Tel. 0272-303030
2. UNIVERSITY OF CAMBRIDGE. "Cellular regulation of proinsulin endopeptidases"  
This project aims to assess the importance of intra-compartmental pH and Ca in the regulation of the endopeptidases involved in proinsulin conversion to insulin in the pancreatic B-cell of normal and prediabetic animals. (Nature 333, 33, [1988]). The successful applicant will become well versed in protein purification methodology, molecular cloning techniques and the immunological approaches to study the biosynthesis, compartmentation and post-translational modification of proteins.  
Applications should be sent to Dr J C Hutton, Department of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge, U.K. Tel. 0223-336790
3. UNIVERSITY OF OXFORD  
NUFFIELD DEPARTMENT OF SURGERY and the INSTITUTE OF MOLECULAR MEDICINE. "Immunoregulation of Type 1 Diabetes"  
The aim of this project is to present Type 1 diabetes in a murine model of the disease to investigate the role of Class II molecules in insulin dependent diabetes and to aim towards a rational approach for the prevention of the disease by manipulation of the pathways in which Class II molecules interact. The successful applicant will be trained in a variety of immunological and DNA techniques.  
Further details are available from Dr J Todd. Tel. 0865-817535. Applications should be sent to The Administrator, The Nuffield Department of Surgery, Level 6, John Radcliffe Hospital, Headington, Oxford OX3 9DU. Tel. 0865-64711 (8585)F

### CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE RESEARCH ASSISTANT/RESEARCH STUDENT

A research assistantship (£10,000-14,000) and a Ph.D. studentship (MRC Scale) are available in the department of Tumour Biology. Applications are invited from candidates who have, or expect to obtain, first or upper second class degree and are highly motivated towards research. The successful applicants will join an active research group working on the control of blood vessel growth in tumours, wound healing and other pathological situations. Applications including a C.V. and the names of two referees should be sent to: Dr D C West, Clinical Research, Christie Hospital & Holt Radium Institute, Wilmslow Road, Withington, Manchester M20 9BX, England. (8598)P

### IMPERIAL COLLEGE (University of London) Blackett Laboratory

#### Postdoctoral Research Assistant

Applications are invited for a postdoctoral research assistantship to work on the interpretation of infrared spectra of Supernova 1987A. Using the Anglo-Australian Telescope, a body of very high quality spectra in the 1 to 4 micron region have been obtained and observations continue. These data are providing unique insights into the structure, composition and evolution of supernovae.

We seek a physicist/astronomer to develop late-time spectral models and to use the results of this work in conjunction with the infrared data to extract physical information about Supernova 1987A. Proven ability in working with astrophysical atomic/molecular spectra and/or radiative transfer processes is desirable.

The post is available for a period of up to three years. Salary is on the RA1A Scale in the range £11,515-£17,370 (including LW) depending on age and experience. Enquiries or applications (full CV and names and addresses of two referees) to Dr. W.P.S. Meikle, The Blackett Laboratory, Prince Consort Road, London SW7 2BZ (Tel. 01-589 5111 X6664).

Closing date for applications: 30 April 1989.

(8547)P

continued on page 31

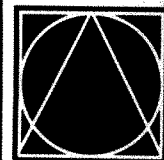


# SMITH KLINE & FRENCH RESEARCH

## Research Donations 1987-88

Smith Kline & French Research are pleased to announce the award of research donations to the listed scientists for innovative investigations:

- Dr C.A.R. Boyd Department of Human Anatomy, University of Oxford  
 Dr K. Burdett Department of Biochemistry and Molecular Biology  
 University of Manchester  
 Professor G. Burnstock Department of Anatomy and Developmental Biology  
 University College, London  
 Dr J.S. Davies Department of Chemistry, University of Swansea  
 Professor A.R. Forrester Department of Chemistry, University of Aberdeen  
 Dr G.F. Gibbons Department of Clinical Medicine, Radcliffe Infirmary Oxford  
 Dr M. Hanley MRC Unit of Molecular Neuroscience, Cambridge  
 Dr J.J. Holbrook Department of Biochemistry, University of Bristol  
 Dr R. Leatherbarrow Department of Chemistry, Imperial College  
 Professor J. Jones Department of Chemistry, University of Surrey  
 Dr O.T.G. Jones Department of Biochemistry, University of Bristol  
 Professor P.M. Nurse Microbiology Unit, Department of Biochemistry  
 University of Oxford  
 Professor R. Ramage Department of Chemistry, University of Edinburgh  
 Dr J.H.P. Tyman Department of Chemistry, Brunel University of West London  
 Dr R.J.K. Taylor Department of Chemistry, University of East Anglia



Smith Kline & French Research Ltd. A SmithKline Beckman Company

(8597)N

GN:ADO278

### MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM RESEARCH AWARDS

Applications are now invited for research awards to enable scientists from the United Kingdom or from abroad to work at the Association's Citadel Hill Laboratory, either as independent visitors or in collaboration with work currently being undertaken as part of the Association's Research Programme. Two levels of award are available:

**RESEARCH BURSARIES** available for visitors at all levels of experience from graduate students to senior scientists. Several awards are made each year with a maximum value of £2,500.

**RAY LANKESTER INVESTIGATORSHIP** available to postdoctoral research workers with several years of research experience in some aspect of marine biology or marine physiology. One award is made each year to a value of £5,000 to enable visitors to work at the Laboratory for a minimum of 5 months over an 18 month period.

Applications are invited for the 1989/90 Financial Year (1 April 1989 to 31 March 1990) with a closing date of 31 March 1989. Enquiries are also welcome for awards to be made in 1990/91. Successful applicants will be expected to become members of the Association.

Further details may be obtained from:

**Dr Gerald Boalch, The Bursar**  
**Marine Biological Association of the**  
**United Kingdom**  
**The Laboratory, Citadel Hill**  
**Plymouth, PL1 2PB, England**

or by telephoning: 0752-609762

(8595)N

### THE ANDERSON PRIZE FOR THE ENRICHMENT OF LABORATORY ANIMAL ENVIRONMENT

At the Silver Jubilee Meeting of the Laboratory Animal Science Association in September 1988, Professor R S Anderson of Liverpool University suggested that in order to encourage the continued improvement of laboratory animal welfare a prize should be given for the best paper published on the enrichment of the environment of animals used for scientific purposes. The Laboratory Animal Science Association and the St. Andrew Animal Fund are pleased to announce that they have jointly taken up this suggestion.

An Annual Open Prize of £750, the Anderson Prize, will be award-

ed for the best original published work which demonstrates a clear benefit to laboratory animals by enrichment of their environment.

The first award will be made for work which has been carried out in the United Kingdom and published during the period January 1st 1983 to December 31st 1988 in scientific or technical journals of any country of origin. Entries by the candidates themselves or by others acting on their behalf, together with four copies of the paper and its English translation if relevant, should be sent to The Anderson Prize, The Laboratory Animal Science Association, 20 Queensberry Place, London SW7 2DZ, so as to arrive not later than July 31st 1989. All entries will be acknowledged. The prize will be presented at the LASA meeting to be held at Solihull in October 1989. (8603)N

### ACCOMMODATION

#### Are you going to the University of Illinois?

Retired U of I professor will exchange house for one abroad for 1989/90. Write **Herbert Goldhor, 39 Maple Court, Champaign, IL 61821, USA.**

(NW3400)S

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the widest international selection of jobs in science — EVERY WEEK

# THE UNIVERSITY OF SHEFFIELD

KREBS INSTITUTE FOR BIOMOLECULAR SCIENCES  
DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY  
**Molecular Biology of Gene Expression**

Applications are invited for a SERC-funded

## Postdoctoral Research Assistantship

tenable for three years starting 1 May 1989 or thereafter.

The project involves biochemical and molecular-genetic studies on the structure, oxygen-sensing, and DNA-recognition of the anaerobic gene activator protein (FNR) of *E. coli*, and it is a joint project between Professor J R Guest and Dr P C Engel.

Applicants, preferably with experience in protein biochemistry or molecular biology should send CV and names of two referees to **Professor J R Guest, Department of Molecular Biology & Biotechnology, The University, Western Bank, Sheffield, S10 2TN** by Friday 23 March 1989, who will supply further details if needed. Starting salary will be up to point 7 on the scale for Research & Analogous Staff, Grade 1A (£9,865-£13,365 pa) depending upon age, and experience. Reference R865/N.

An Equal Opportunity Employer

(8573)P

## COURSES & CONFERENCES & SYMPOSIA

### HARDEN CONFERENCE

#### 32nd: 'INOSITOL LIPIDS IN CELL FUNCTION'

Wye College, Ashford, Kent, 3-8 September 1989

Organizing Chairman: R. H. Michell (Birmingham)

Harden Lecturer: M. J. Berridge

*Principal Topics:* Receptor and G-protein coupling to phosphoinositidase C; Molecular Biology of phosphoinositidase C and protein kinase C; Calcium mobilisation and other functions of inositol phosphates; Relationship between inositol lipid signalling, cell proliferation and oncogenes; Inositol lipid glycans in protein anchoring and cell regulation.

*Conference Fee and Full Board (inc. VAT):* £250 for applications received by 1 June 1989, £300 for those received thereafter.

*Information:* The Meetings Officer, The Biochemical Society, 7, Warwick Court, London, WC1R 5DP, U.K. Tel: 01-242 1076, FAX: 01-831 1853. (8590)C

### IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE (University of London)

#### M.Sc. in Chemical Research

This one-year degree course provides training in modern research methods and includes an individual research project in a branch of chemistry. Applicants should hold (or expect to hold) a good honours degree in chemistry or a combined subject degree with chemistry as one component.

Further details and application forms may be obtained from the **Senior Assistant Registrar (Admissions), Imperial College, London SW7 2AZ.** (8587)C

### HARDEN CONFERENCE

#### 33rd: 'CELLULAR BARRIERS AND DRUG TARGETTING' Wye College, Ashford, Kent, 10-15 September 1989

Organizing Chairman: H. M. Patel (London)

Harden Lecturer: G. E. Palade

*Principal Topics:* Physiological and biochemical properties of cellular barriers; Anatomical and cellular considerations in drug targetting in liver and lung; Serum opsonins and Reticuloendothelial system; Drug carriers; Clinical trials with drug carriers; Specific delivery via nasal, gastrointestinal, systemic, transdermal, intracellular routes.

*Conference Fee and Full Board (inc. VAT):* £250 for applications received by 1 June 1989, £300 for those received thereafter.

*Information:* The Meetings Officer, The Biochemical Society, 7 Warwick Court, London, WC1R 5DP, U.K. Tel: 01-242 1076, FAX: 01-831 1853. (8591)C

Sheffield City Polytechnic  
Department of Biomedical Sciences

## 1989 Short Course Programme

Wednesday 10-Friday 12 May

### Which Immunoassay?

Faced with an ever increasing range of methods for immunoassay, the busy laboratory scientist is often hard pressed to make a choice. By combining informative lectures and workshops, the participants will investigate RIA, IRMA, EIA and 'dedicated' systems, gaining hands-on experience on which to base an answer to "Which Immunoassay?"

Fee: £195.00

Monday 12-Friday 16 June

### The Intensive Radioimmunoassay Course

Once again, we are pleased to offer this popular and informative course covering all aspects of RIA and associated techniques, from first principles to latest developments in the field. An intensive course of lectures and practical exercises will ensure that participants have a thorough background of the technique.

Fee: £295.00

Wednesday 5-Friday 7 July

### Recombinant DNA Technology — A Refresher Course

Recombinant DNA technology is one of the areas of growth in modern research. By a combination of lectures and demonstrations, this course will update workers in the field, and keep them abreast of the latest developments.

Fee: £125.00

Monday 10-Friday 14 July

### Modern Techniques in Enzyme Immunoassay

Enzyme linked immunoassay is a widespread technique, and forms the basis of many commercially available analysis protocols. By combining lectures with a rigorous practical schedule, participants will investigate enzyme immunoassay techniques using model and real systems and evaluate many of the variants of the basic technique.

Fee: £295.00

Other course planned for later this year are

- Everyday Molecular Separation
- Modern Applications of UV/VIS Spectrophotometry

Further details on these courses may be obtained from:

Short Course Support Unit Quoting ref 229

Sheffield City Polytechnic

43 Broomgrove Road

SHEFFIELD S10 2NA

Tel: (0742) 665274 Ext 3273

(8541)C

Polylink



Sheffield City Polytechnic

FIRST ANNOUNCEMENT, CALL FOR POSTERS AND REGISTRATIONS



**FOURTH INTERNATIONAL  
CONFERENCE ON PROGRESS  
IN CANCER RESEARCH  
APRIL 30 – MAY 2, 1989  
SAN REMO, ITALY**

**Organized by:**

National Institute for Cancer Research – Genoa, Italy  
Italian League Against Cancer

**In collaboration with:**

Regione Liguria  
Faculty of Medicine University of Genoa, Italy  
Imperia Provincial Council, Italy  
The City Council of San Remo, Italy

**Scientific and Organizing Committee:**

Renato Dulbecco, U.S.A. (Chairman); Robert C. Gallo, U.S.A.;  
Lorenzo Moretta, Italy; Leonardo Santi, Italy; Inder M. Verma, U.S.A.

**General Secretary:**

Giovanni Lotti, Italy

**Conference topics will focus on:**

Oncogenes and Antioncogenes – Lymphocyte Subpopulations – Cell  
Signalling Lymphokines – Anti-Tumor Cytotoxicity – Immunotherapy  
of Cancer – AIDS and Cancer

**Invited participants include:**

Anton Berns, the Netherlands; Luigi Chieco-Bianchi, Italy; Giuseppe  
Della Porta, Italy; Renato Dulbecco, U.S.A.; Ronald M. Evans, U.S.A.;  
Manlio Ferrarini, Italy; Guido Forni, Italy; Luigi Frati, Italy; Robert C.  
Gallo, U.S.A.; Felice Gavosto, Italy; Thomas Graf, Germany; Carlo  
Grossi, Italy; Edward Harlow, U.S.A.; Michael Y. Hawkins, U.S.A.;  
Chris Y. Marshall, England; John D. Minna, U.S.A.; Malcolm A. S.  
Moore, U.S.A.; Alessandro Moretta, Italy; Lorenzo Moretta, Italy;  
Sergio Romagnani, Italy; Sandro Pontremoli, Italy; Elio G. Ronda-  
nelli, Italy; Gaetano Salvatore, Italy; Leonardo Santi, Italy; Jack L.  
Strominger, U.S.A.; Jean Paul Thiery, France; Giancarlo Vecchio,  
Italy; Inder M. Verma, U.S.A.; Carlo Zanussi, Italy; Luciano Zardi, Italy.

**Registration fee, including lunch, will be 400,000 Italian Liras (about  
300 US Dollars). A limited number of prizes covering the registration  
fee and the expenses for Hotel accommodation will be awarded for  
selected abstracts.**

**Application forms and abstracts should be submitted before March  
10, 1989 to:**

Servizio Attività Culturali Istituto Nazionale per la Ricerca sul Cancro  
Viale Benedetto XV, 1016132 GENOVA, Italy (W5893)C

**Call for Papers**

**EIGHTH INTERNATIONAL OCEAN DISPOSAL  
SYMPOSIUM (8IODS)**

**Dubrovnik, Yugoslavia — 9-13 October 1989**

The overall objectives and scope of 8IODS are:

- Provide a forum for exchange of ideas and information among investigators involved in marine pollution and oceanic disposal research
- Enhance the scientific consideration of waste disposal and marine pollution processes.
- Generate recommendations and guidelines for future studies of oceanic disposal practices.

The 8IODS will feature special sessions and invited keynote speakers. The Co-Chairman of 8IODS are: Dr. Velimar Pravdic, Ruder Boskovic, Zagreb, and Dr. Adam Benovic, Biological Institute, Dubrovnik, Yugoslavia.

Send 250-word abstract by 31 May 1989 to:

**Dr. Iver W. Duedall, Organizing Committee Co-Chairman,  
Department of Oceanography and Ocean Engineering,  
Florida Institute of Technology, Melbourne, Florida 32901  
USA. (NW3381)M**

**Molecular Communication in Higher Plants**

**18-21 September 1989**

**at EMBL, Heidelberg, F. R. Germany**

**Invited speakers include:**

D. Baulcombe (GB-Norwich), M. Bennett (GB-Kew), M. Bevan (GB-Cambridge), T. Bisseling (NL-Wageningen), U. Bonas (D-Berlin), C. Bowler (B-Gent), F. de Bruijn (D-Köln), M. Caboche (F-Versailles), N.-H. Chua (USA-New York), A. Clarke (Australia-Melbourne), E. Coen (GB-Norwich), G. Coruzzi (USA-New York), J. Dangi (D-Köln), E. Dennis (Australia-Canberra), A. Gatenby (USA-Wilmington), W. Gerlach (Australia-Canberra), A. Gierl (D-Köln), R. Goldberg (USA-Los Angeles), W. Gruissem (USA-Berkeley), R. Hedrich (D-Göttingen), T. Hohn (CH-Basel), R. Horsch (USA-St. Louis), D. Jofuku (B-Gent), J. Jones (GB-Norwich), J. Leemans (B-Gent), P. Meyer (D-Köln), D. Mohnen (USA-Athens), T. Nelson (USA-New Haven), K. Palme (D-Köln), I. Potrykus (CH-Zürich), P. Quail (USA-Albany), E. Schäfer (D-Freiburg), A. Sievers (D-Bonn), C. Somerville (USA-East Lansing), A. Spena (D-Köln), D. van der Straeten (B-Gent), A. Trewavas (GB-Edinburgh), S. C. de Vries (NL-Wageningen), E. Weiler (D-Bochum), P. Weisbeek (NL-Utrecht), U. Wienand (D-Köln), L. Willmitzer (D-Berlin).

**There will be nine plenary sessions which will cover:**

Cell-cell interactions; Differential gene expression/mutational analysis of plant function; Inter and intracellular signalling and the regulation of plant growth; Plant and environment; Plant biotechnology; Plant and microbes; Plants and viruses; Protein traffic and assembly into higher order structures; Structure, modification and expression of the nuclear genome (including novel techniques for gene isolation). The two poster sessions will allow participants to present their work.

**Registration:**

The Symposium will be at the European Molecular Biology Laboratory, Heidelberg, with registration on Sunday, 17 September 1989. The registration fee, which includes daily transport to and from the EMBL, lunches, and the Symposium reception, is DM 180, for graduate students DM 90, and for participants from industry DM 360. Participants will be accommodated in the EMBL guest house and hotels in Heidelberg. The registration fee does NOT cover the cost of accommodation.

**Application:**

The deadline for applicants is 16 June 1989. Applications should include a curriculum vitae and a brief description of research interests. The organizing committee will notify those who have been accepted as soon as possible after the deadline. The total number of participants will be limited to 250. Applications should be addressed to **Dr. J. Tooze, EMBO, Postfach 102240, D-6900 Heidelberg, F. R. Germany**. Applicants wishing to present a poster should send a 1-page abstract together with the registration fee and reply sheet after they have been accepted for participation.

**Organizing committee:**

C. Leaver (Edinburgh) –Chairman–, K. Marcker (Aarhus), M. van Montagu (Ghent), I. Potrykus (Zürich), H. Saedler (Köln), F. Salamini (Köln), J. Tooze (EMBO, Heidelberg). (W5907)M



**COLD SPRING HARBOR LABORATORY  
CONFERENCE ON  
YEAST CELL BIOLOGY**

**AUGUST 15 - 20, 1989**

**Organized by:**

Scott Emr, *California Institute of Technology*  
Amar Klar, *NCI, Frederick Cancer Research Facility*  
John Pringle, *University of Michigan*  
Steve Reed, *Scripps Clinic & Research Foundation*

**Topics to be covered will include:**

- Cell cycle controls
- Developmental choices
- Cell-cell recognition
- Cytoskeleton and cell structure
- Organelle structure and function
- Protein targeting and modification
- Elements of chromosome structure and function

**The abstract deadline is May 31, 1989.**

**Registration & abstract materials may be obtained from:**

**Meetings Coordinator**

**Cold Spring Harbor Laboratory**

**Cold Spring Harbor, NY 11724**

**516-367-8346 FAX 516-367-8845**

(NW3402)C



**1989**

# PHILIPPE LAUDAT CONFERENCES

**LE BISCHENBERG • OBERNAI**

## ★ Human mitochondrial diseases : clinical features, biochemistry and molecular genetics of oxidative phosphorylation defects

**★ OCTOBER 1-5 1989**

### Preliminary Programme :

Mitochondrial DNA : function, maternal heredity, deletions, mutations

Defects in complexes I, III and IV of the respiratory chain

New investigative methods for mitochondrial cytopathies

Strategy for therapy

Animal models for mitochondrial myopathies.

## ★ The neuroendocrine-immune network : molecular aspects ★ OCTOBER 15-19, 1989

### Preliminary Programme :

Heterologous actions of hormones and cytokines

Neuromediators and immune function

Neuroendocrine peptides and immunity

Hormones and immunity

Cellular origin and functions of « ectopic » signals

Signals transduction

Antigenic mimicry

Integrated topics : clinical correlates.

## ★ Bacterial pathogenesis, from molecular genetics to cell biology

**★ OCTOBER 22-26, 1989**

### Preliminary Programme :

Bacterial genetics : new tools to study pathogenesis

Bacterial attachment, phagocytosis, cellular traffic

Bacterial invasion, movement of cell organelles

Bacterial toxins, G-proteins, cytoskeleton.

Posters sessions are also planned for each conference.

Scientists wishing to attend these conferences can obtain informations and application forms at the following address :  
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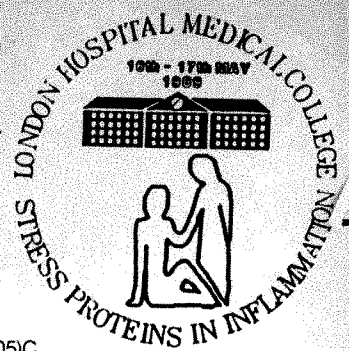
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(W5929)M

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(W5826)M



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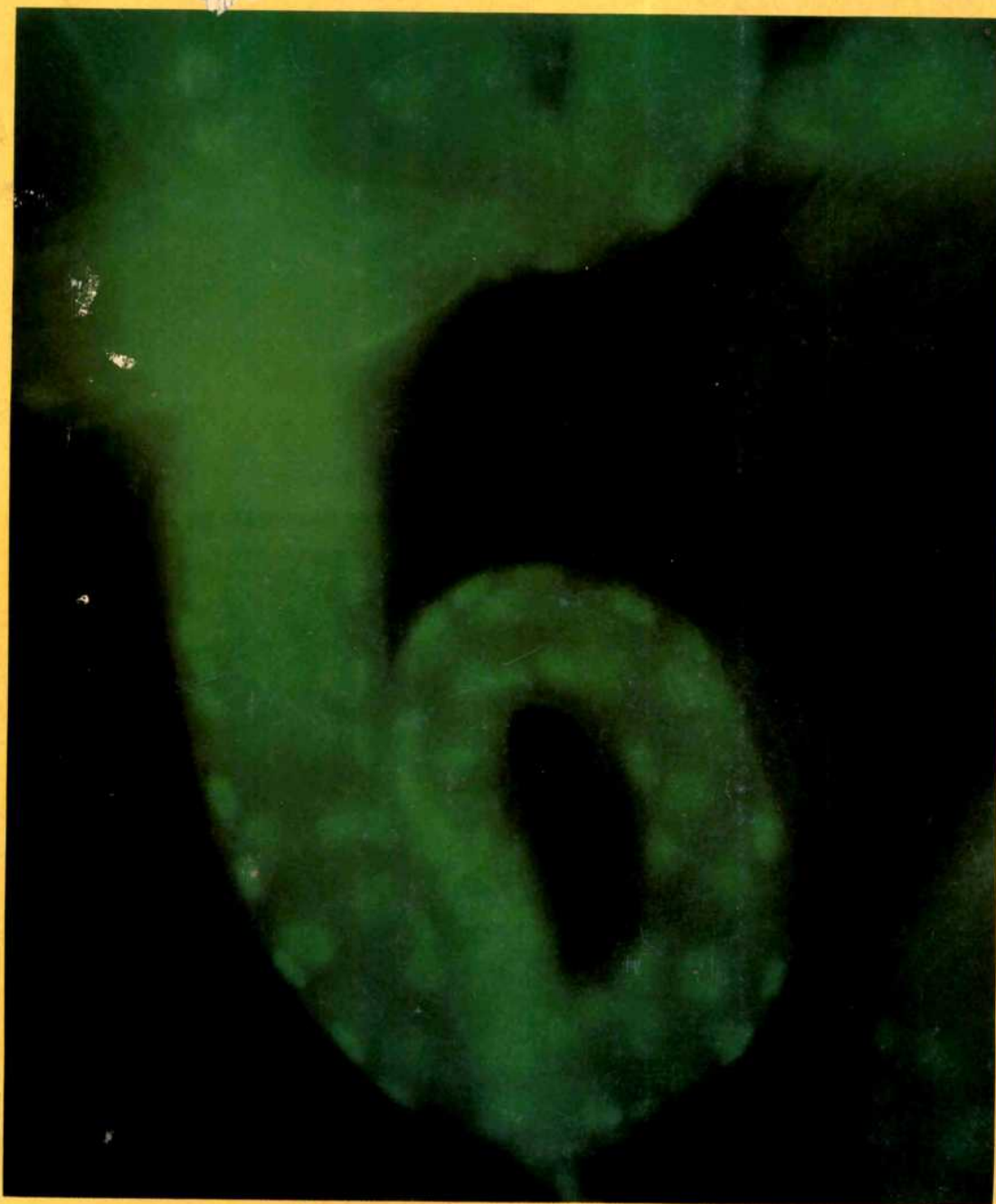




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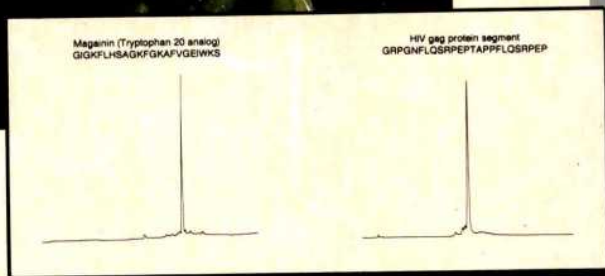
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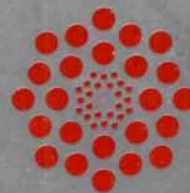
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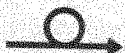
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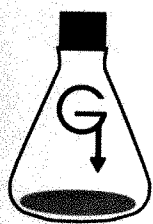
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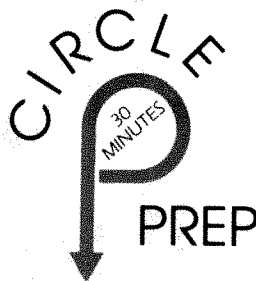
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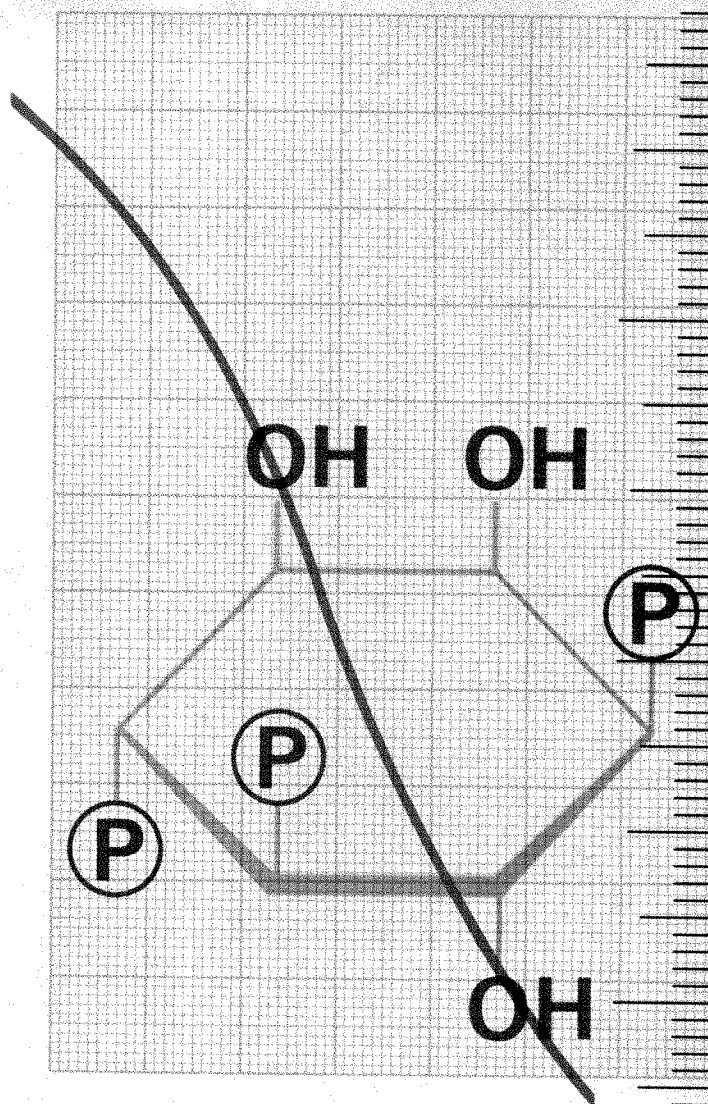
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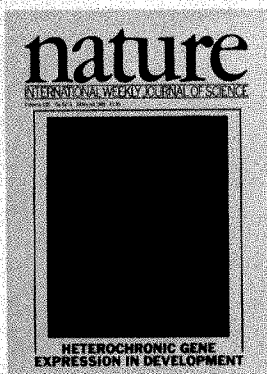
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# nature

23 March 1989

Vol. 338 Issue no. 6213

The cover illustrates the use of immunofluorescence staining to locate the product of the heterochronic gene *lin14* in an embryo of the nematode *Caenorhabditis elegans*. The gene encodes a 'temporal development switch', as described in the Article on page 313.

## THIS WEEK

### Scrapie-like syndrome

Gerstmann-Sträussler syndrome, a rare neurodegenerative disease with infectious properties that can also be inherited, is associated with a very rare amino acid substitution in the 'prion' protein, page 342. And see 'Sheep disease in human clothing', page 298.

### Synthesis and use

Workers from AT&T Bell Laboratories describe a method of synthesizing the '124' superconductor without the need for specialized equipment, page 328. On page 330, an application for high- $T_c$  superconducting ceramics in the separation of paramagnetic and diamagnetic molecules.

### Brain waves

Oscillatory responses of widely separated neurons in the visual cortex of the cat are shown to be synchronized if the cells have the same orientation properties. This may be a mechanism for establishing relationships between stimuli in different parts of the visual field, pages 334 and 297.

### Voyager's best shot

The highest-resolution image from Voyager II of Uranus's moon Umbriel (below) shows a dark, heavily cratered surface. Photometry data suggest that present



surface patterns are the result of tectonic disruption early in the lifetime of the moon, page 324.

### Pulsar in theory

The odd properties of the recently discovered pulsar in supernova 1987A have provoked competition among theoretical astrophysicists. Is the neutron star not rotating but vibrating (page 319)? If it is rotating, was it born spinning 2,000 times a second, or has it been spun up by accretion (page 321)? For a sober assessment of problems and possibilities, see page 295.

### Cyclins A and B

Cyclins are proteins that vary in concentration with the cell cycle, rising until mitosis then falling sharply. Of two cyclins in the fruit-fly *Drosophila*, maternal mRNA for one becomes localized in the pole cells destined to form the germ line of the developing fly. This cyclin B may have a specialized function in regulating germ-cell proliferation, page 337.

### Chromosome probes

Chromosome 8 (arrowed), target



for a microdissection technique for isolating probes for defined regions of the human chromosome. Page 348.

### Atmospheric pollution

Measurements taken at the mountain-top Mauna Loa Observatory in Hawaii point to fossil fuel burning in the United States as a source of soluble atmospheric nitrogen over the island in summer and autumn. Page 326.

### Author Index

Facing page 360 this week is the Author Index for Volume 337, including a Guide to Authors.

## NATURE SAYS

Bovine growth hormone is an unworthy cause of US/European dispute ■ Productivity the only cure ■ British academics' strategy for pay

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## NATURE REPORTS

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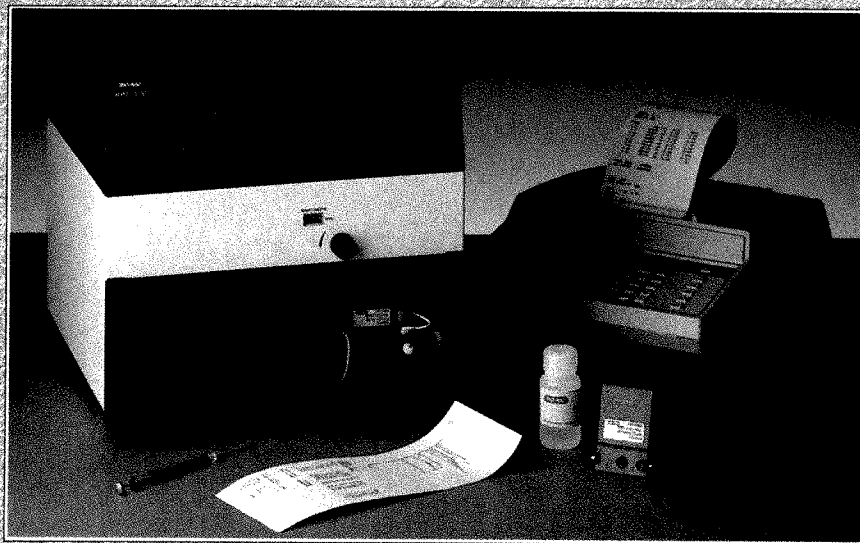
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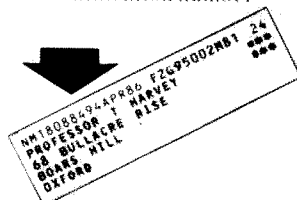
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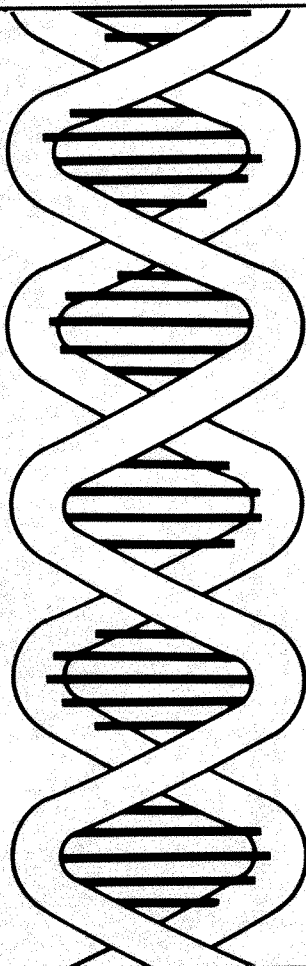
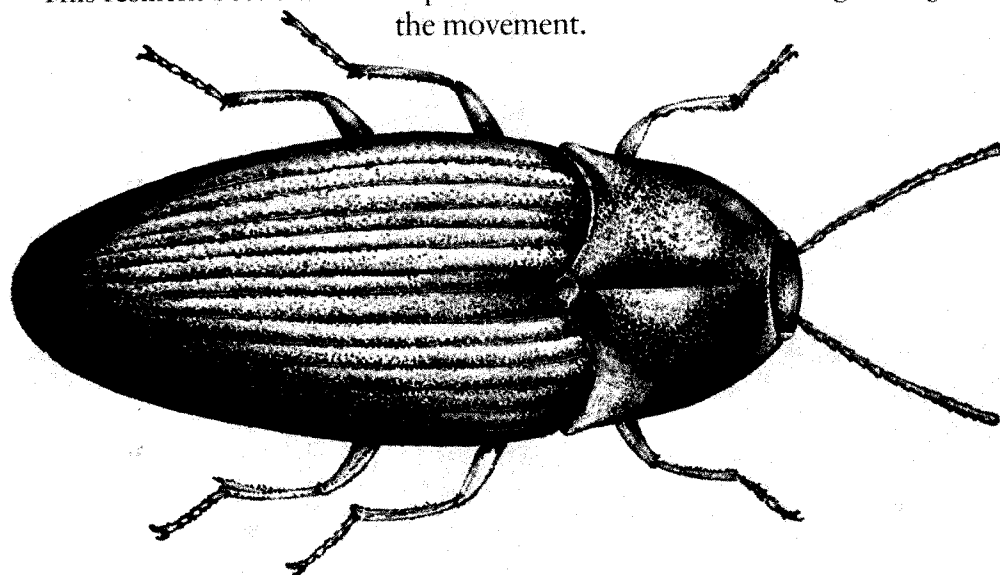
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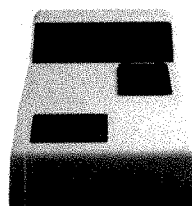
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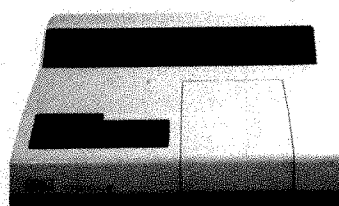
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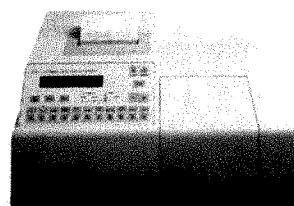
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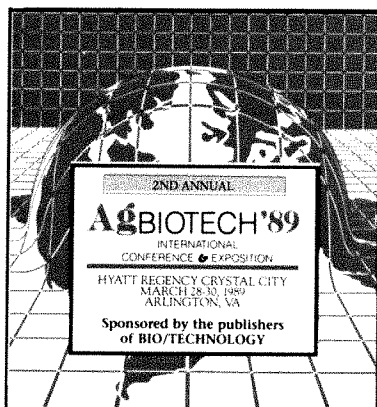
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8:00 a.m. – 11:00 a.m.

Management Strategies  
 Market Sizes and Structures  
 Long Range Strategic Planning

Chair: David Wheat  
 Panelists: A. Barnes, G. Kidd, R. Laster

11:00 a.m. – 2:00 p.m.:

2:00 p.m. – 5:00 p.m.

Management Strategies  
 International Strategic Alliances  
 High Level Staffing

Chair: Kelly Kincannon  
 Panelists: C. Baker, J. Bouckaert, J. Marcus

Tuesday, March 28

8:00 a.m. – 11:00 a.m.

Regulation: Case Studies; Testing for Registering Microbial Pesticides

Chair: Edgar R. Butts and Robert B. Nicholas  
 Panelists: D. Glass, C. Hutchinson, R. Kahn, J. Panetta, F. Serdy, J. Swigert, S. Woodhead

11:00 a.m. – 2:00 p.m.:

2:00 p.m. – 5:00 p.m.

Commercial Financing

Chair: G. Steven Burrill  
 Panelists: C. Earl, R. Moshe, L. Stern, D. Wagster

Wednesday, March 29

8:00 a.m. – 11:00 a.m.

Patents: Animal; Plant

Chair: Kathleen Merrigan  
 Panelists: D. Beier, A. Douglas, J. Doyle, H. Lyman, L. McKenzie, K. O'Conner, R. Quisenberry

11:00 a.m. – 2:00 p.m.:

1:30 p.m. – 4:30 p.m.

Government Research Funding Policies

Chair: Gary B. Ellis  
 Panelists: R. Dull, V. Giddings, K. Hanna, W. Marshall, M. Phillips, J. Tavares

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Plant Molecular Biology		Animals	
8:00 a.m. – 11:00 a.m.		8:00 a.m. – 11:00 a.m.	
The Production of Transgenic Crops Dicots – Cotton, Soybean, Oil Seed Rape, Walnut Pine  Chair: Harvey Bialy Panelists: K. Barton, A. Dandekar, J. Fillatti, M. Hinchee		Molecular Strategies for Production Animal Improvement: Transgenes, Genome Mapping  Chair: Charles Arntzen	
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS			
2:00 p.m. – 5:00 p.m.		2:00 p.m. – 5:00 p.m.	
The Production of Transgenic Crops Monocots – Rice, Maize, Asparagus  Chair: Indra Vasil Panelists: R. Shillito, M. Von Montagu, R. Wu		Molecular Strategies for Production Animal Improvement: Transgenes, Genome Mapping (continued)  Chair: Charles Arntzen	

Tuesday, March 28

Perspectives		Biopesticides	
8:00 a.m. – 11:00 a.m.		8:00 a.m. – 11:00 a.m.	
Nitrogen Fixation  Chair: Ethan Signer Panelists: D. Kahn, D.P. Verma, G. Walker		Biopesticides Bacillus thuringiensis Endophytes Baculovirus  Chair: Jerry Caulder	
EXHIBIT HALL, LUNCH, EXHIBITOR WORKSHOPS			
1:30 p.m. – 4:30 p.m.		1:30 p.m. – 4:30 p.m.	
The International Picture  Chair: Mark Ratner Panelists: J. Cohen, P. Oram, W. Roca, M. Sondahl, G. Toenniessen		Biopesticides (continued)  Chair: Jerry Caulder	

Thursday, March 30

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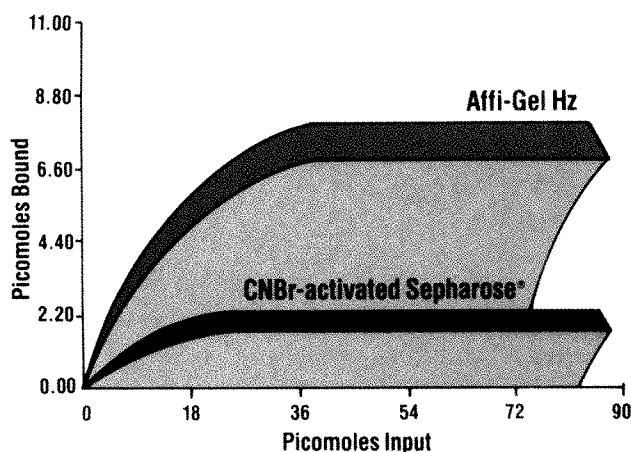


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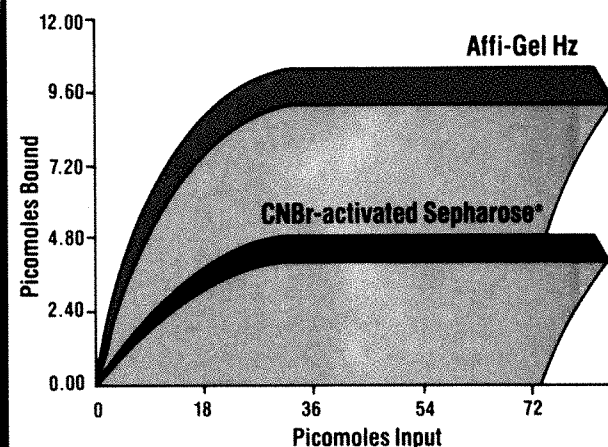


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## More wicked ways with hormones

The European Commission has hung an intellectual millstone around its neck by banning the use of bovine growth hormone in growing cattle. The European Parliament has a duty to put it straight.

VISIONARIES who proclaim that the United States of Europe is no further away than 1992, when the European Economic Community will constitute the single market originally decreed by the Treaty of Rome, habitually overlook the still-glaring differences between Europe and the United States. True, Europe has a president (now M. Jacques Delors), but does not require that he should be elected as Mr George Bush has been. And while the members of the European Commission (now 17 strong) are roughly analogous to members of a cabinet, they too are merely nominated every four years by the political heads of member governments. There is also a single-chamber European Parliament, the equivalent of the US Congress, which does suffer periodic re-election (next in June this year), but the opinion even among ardent Europeans is that the parliament will have to win influence in European affairs by demonstrating its value and good sense. The same Europeans will therefore be dismayed by one development last week — the decision of a parliamentary committee to support and even to reinforce the regulations that prohibit the use of bovine growth hormones in the fattening of cattle.

The circumstances are quite scandalous. Since the beginning of 1988, the sale in Europe of beef grown with the use of bovine growth hormone has been prohibited by a directive (with the force of law) of the European Commission. Although the measure has been justified as a means of protecting people's health, there is literally no evidence that beef produced with growth hormone is a hazard to health, but every reason to believe that bovine growth hormone in cattle is rapidly metabolized (which is why it helps cattle to grow more quickly). So why ban its use? Because Europe's system of support for agriculture is a means of over-producing all kinds of commodities, beef included, and because those administering the system were ready to clutch at any straw to prevent further surpluses of beef. What more natural than an alliance with the green extremists to keep the hormone out of use? Although imports of beef from the United States were exempted from restriction during 1988, this year began with an acrimonious trade dispute between Europe and the United States that rumbles on.

Now the European Parliament has entered the fray, but discredibly. Last week its environment committee in Luxembourg issued the gist of a report supporting the European Commission's ban and urging more vigilance in

tracking down now-illicit uses of these materials. The opportunity for extricating the Commission from the pit of unreason it had dug for itself has been spurned in the face of informed opinion and despite the diplomatic damage the ban has already caused. In the short run, the consequence will be more trouble. Unless the full parliament rejects the opinion of its committee in the discussion arranged for April, it will find that in the long run, it has much less influence than it might have won by acting sensibly, which should worry ardent Europeans, green and not-so-green. □

## Budgets off balance

Budget problems in Britain, the Soviet Union and United States may have different origins — but one solution.

AMONG the quaintest British customs is the annual ritual in which a person called the Chancellor of the Exchequer (now Mr Nigel Lawson) tells the House of Commons what taxes he plans to increase or decrease. The procedure is the inverse of what happens in most other places, where a government's annual budget is a statement of what it plans to spend coupled with a comparison of total spending and total revenue. In Britain, the spending estimates appear first, as fiat. That is why Lawson's performance last week (his sixth) has generally been written off as "dull" for, breaking with recent form, he offered neither tax breaks nor further imposts, except marginally.

But there is more than that to say. The British government is in the enviable position that its accounts are in surplus. In the year ending this month, there will be a surplus of £14,000 million. With the over-cautious forecast that next year's surplus will be the same, the British government could liquidate its entire debt within a decade. But is there nothing better to do with surplus funds than pay off debts? Not on British experience in the past few years. Putting more money in British pockets (either by reducing taxes or spending money) seems either to increase imports or industrial costs (through higher wages) — or both. Britain already has a trade deficit comparable with its budget surplus and slightly larger (as a percentage of Gross Domestic Product) than that of the United States. And inflation is pushing 8 per cent, so that interest rates have increased and sterling has

been allowed to increase in value against other European currencies. Lawson made a clean breast of his dilemma last week: despite the £14,000 surplus, he had no room for manoeuvre.

Notoriously, exactly the same is true in the United States, where the Deficit Reduction Act (better known as Gramm-Rudman after two of its three sponsors) puts a statutory and reducing ceiling (to be fixed finally only in the summer) on the federal budget deficit. Again, the government has no room for manoeuvre. Part of the trouble is that more than a third of US spending is fixed by various acts of Congress, while just under a third goes on defence. Because savings in the United States are not great enough to bridge the gap, the deficit has been financed by borrowing from elsewhere (which is why there is a trade deficit). Over the past 18 months, two devices have been used to correct the balance — interest rates have been increased (as in Britain) but the value of the US dollar has been allowed to decline. This house of cards depends on people's willingness to continue turning foreign currencies into dollars to bridge the gap; ideally, the lenders would have been given proof of a deal between the administration and the Congress early in the new presidency, but even optimists are now talking of cutting a deal only in the summer. The sharp if modest fall of the stock markets last week, ostensibly from fears of still higher interest rates, may be a sign that the delicate apparatus has begun to crumble.

Mr Mikhail Gorbachev is similarly hemmed in. The Soviet Union's budget is believed to be 20 per cent in deficit, but the consequent inflation is only marginally apparent because most prices are centrally controlled. Because there is so little on which people can spend money, they save it against the time when the shops are full again. Last week, Gorbachev outlined an agricultural reform that would have seemed revolutionary before he came to office; farmers are to be allowed personally to lease the land they farm on the grounds that they will then become more efficient, but the details are not yet made public and are probably still to be decided. One snag, not nearly as well advertised, is that even more efficient farmers will not grow more food unless prices are also increased, perhaps even by a free-market mechanism. Sadly, even the bare bones of the scheme will give ideological offence.

Why should three substantial governments be thus hamstrung? To compare the United States and the Soviet Union economically is absurd, but that is not the end of it. The United States is locked into a straitjacket because, despite the astonishing flexibility of its economy and the steady growth of production, expectations have stolen a march on real wealth. President Bush's hope, like his predecessor's, is that the United States will grow out of trouble, but even the present modest rate of growth is judged dangerously fast. The safer short-term remedy is modestly to reduce consumption, which the new president has promised he will not, but in the long run only an increase of productivity (rather than of mere production)

will square the circle. Exactly the same applies in the Soviet Union, but should be more achievable, given both the encouraging and the admonitory examples with which the world is littered. And, as it happens, the same long-term remedy is what Britain should also be looking for. The box in which Lawson is imprisoned would be less constraining if it were possible to spend (or give away) some or all of that budget surplus without rocking the boat. Maybe the three people chiefly concerned should put their heads together. □

## Universities in arms

British academics are faced with a nasty choice between a poor pay award and a continuing labour dispute.

THE reasons for believing that British academics are underpaid do not strain the imagination. Salaries have grown less quickly than those in other professional walks of life over the past decade of general deprivation for universities, and now compare so much less well than those in industry and elsewhere that university teachers and researchers are on the march to other jobs. The more serious worry is that recruitment to academic research at all levels is quickly drying up. Even school-leavers have learned that the academic profession is not much valued by the academic system's paymasters. Since the beginning of this year, academics have been declining to mark examination papers; now they have to choose between the continuation of that fruitless pursuit and their employers' offer of a salary increase of 6.5 per cent (which for practical purposes covers a two-year spell). The best course is to settle for what is on offer, but to prepare to fight a better battle next year.

The world, by now, knows that the vast majority of universities do not have the funds with which to pay more than they have offered. The whole world should also know that the British system cannot continue as in the past without running into serious difficulties. Hitherto, British academic pay has been determined nationally, by negotiation between the Association of University Teachers and the Committee of Vice-Chancellors and Principals. But the British university system is now well on the way to a welcome diversity in which some universities will be more able than others to pay good salaries. Disparities can only be further accentuated if the British government decides that the time has come to increase, perhaps double, the tuition fees paid from public funds to the universities which students attend, but recovering the cost from running budgets. Then universities will be competing tooth and nail for students.

Whether academics (or their union) welcome this development, it is happening and will continue. Nationally negotiated pay awards are bound eventually to disappear. It would be sensible to anticipate that certainty, if only for the sake of the greater say that academics would thereby acquire in the management of their own institutions. □



# Soviet Academy faces grass-roots revolt

- Academy's official candidates at risk
- Democratic fervour takes a hold

## Moscow

THE nomination conflict at the Academy of Sciences of the USSR is building up to a dramatic denouement this week — the academy's list of candidates may even be voted down by the academy's research institutes. The conference at which the academy will elect 20 of the 23 nominated candidates was scheduled for 20–22 March, at the Youth Cultural Centre in Moscow. The voters will be the 907 full and corresponding members of the academy and 440 institute representatives.

Since the January meeting at which Academicians Andrei Sakharov and Roald Sagdeev were voted down despite the recommendation of many institutes, ordinary scientists have seen the outcome as a blatant disregard of their opinions. After the protest meeting outside the academy on 2 February (see *Nature* 337, 593; 1989), several institutes formed a group to press for democratic elections.

Now the group has held a meeting of Moscow-region delegates to this week's electoral conference at which it was agreed to vote against all 23 official nominees unless the list is abrogated or enlarged (but enlargement is no longer possible). The Moscow region accounts for nearly half of all academy scientists.

The meeting emphasised that its intended "No" vote is not directed against particular nominees but is a protest against the "list imposed on the academy conference despite public opinion".

Even so, the outcome may be dramatic. Under the election rules, candidates who

fail to secure at least one vote more than half of the total eligible to vote will not be elected. If the number of successful candidates is fewer than 20, the conference will have to nominate further candidates on the spot, which is what the protesters want. Much will depend on the full and corresponding members of the academy, who will have a majority this week.

Will the academy bosses allow those with fewer letters after their names to carry the day? The answer cannot be simple. Not only the election is at stake, but the other issues triggered by January's nomination meeting, which in retrospect is the stone that started an avalanche of reforming public opinion in science.

Democratic reform is sweeping the academy. The procedure of electing institute, department and laboratory heads has been changed beyond recognition. Research groups compete for research grants, which is a novel development. Think tanks are set up for particular problems in research and development.

But the outdated is stubborn and tenacious, even though the backward-looking do not offer open resistance. Moth-eaten patterns of behaviour have inertia, and the research community is wary of risky ventures.

The protest meeting at which the decision was taken to vote "No" against the official list of candidates was also the first to voice a now-popular idea — that of creating an independent Soviet scientists' union. The praesidium of the academy supported this initiative at its meeting of 7 March, and appointed a working-group under Yuri Osipyan, an academy vice-president and director of the Moscow Solid State Physics Institute, to study the idea; a national researchers' conference is planned.

But the inter-institute activist group is in a ferment, regarding the academy's action as a means of appropriating its own project and of attaching the budding union to the praesidium.

At a rally of Moscow researchers which I attended on 11 March, the union's prospects were debated. Although the organizers had invited members of the academy's upper echelons, the bosses were conspicuous by their absence. The rally adopted the text of an address to Soviet scientists about the formation of the union, and elected an organizing committee with close on 40 members. An inaugural conference is expected in May.

**Yuri Kanin**

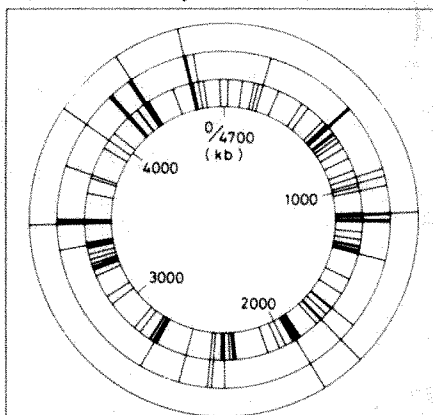
*Nature Moscow correspondent/Novosti*

# Full sequence for *E. coli*

## Tokyo

JAPAN'S Ministry of Education, Culture and Science (MESC) will shortly announce its backing for a project to sequence the entire genome of the bacterium *Escherichia coli*. With luck, *E. coli*'s estimated 4,700 kilobase pairs, one thousandth the number of the human genome, could be sequenced in five years. Funding for the project will remain uncertain until the Diet passes the 1989 budget, delayed by political quarrels over the Recruit bribery scandal. But given the scale of MESC's 'priority areas of research' fund, it seems likely that more than a million dollars a year will be provided for an initial three years.

The project is led by Takashi Yura of Kyoto University and Katsumi Isono of



By July 1987, most of the *E. coli* genome had been cloned, as shown in the outer ring here. The bars represent unclosed regions and the inner two rings show the 'gaps' closed by successive clonings. Scale in kilobases from 0 min. the *thr* locus. (Modified from Y. Kohara, K. Akiyama and K. Isono, *Cell* 50, 495; 1987.)

Kobe University and builds on a physical map of the *E. coli* chromosome put together from 3,400 clones (see box). Researchers from Kyoto, Kobe, Osaka and Tokyo universities will make up the core of the project, but international collaboration should become possible once a clone bank has been established at the National Institute of Genetics at Mishima.

Of course, *E. coli* has already been extensively studied. More than 1,000 genes have been mapped and about 450 kilobase pairs have been sequenced by researchers around the world. Isono says he hopes his laboratory alone will be able to manage 200–500 kilobases a year. He adds a note of caution, however, because in sequencing projects, "90 per cent of the work is done in 50 per cent of the time", with unbridgeable gaps remaining.

If successful, the complete *E. coli* sequence will be the first for an independently living organism.

**Alun Anderson**



Moscow spring: thousands marched in Moscow in support of pro-perestroika Boris Yeltsin in Sunday's election.

# California may take hard line

## Berkeley

THE University of California (UC) is contemplating changes to its tenure policy for faculty members that would permit the removal of the "grossly incompetent". The new policy was approved by the Berkeley faculty senate last month; other campuses are considering adopting the same rules. Supporters of the new measure say it provides clear and specific criteria by which to judge faculty performance, specifying as it does dismissal rather than demotion for those judged incompetent.

US universities have been forced to tackle the issue of faculty competence, especially where older people are concerned, because federal law may soon prohibit compulsory retirement ages. Indeed, universities were exempted from the federal Age Discrimination in Employment Act of 1984, which made mandatory retirement illegal, and allowed to require retirement at 70, but that exemption will lapse in 1993 if not renewed by Congress.

Out of a general concern over "non-productive faculty", UC began in 1984 to review all tenured faculty members who had not appeared before a promotions or other committee for at least five years. During the first round of reviews, five people were found to be both creatively unproductive and grossly deficient in teaching.

While university rules allow that tenure may be terminated "for good cause", the

service to students". Faculty members whose five-year evaluations produced such a record would be offered counseling and assistance, such as retraining, says English professor Ralph Rader, who presented the proposal to the academic senate last month. Only if those failed would dismissal be considered.

The purpose of the proposal is to set up machinery for dealing with incompetence that is so formalized and specific, says Rader, that it will automatically be set in motion by a negative review. But he expects that most such findings would lead not to unconditional dismissal, but to negotiated early retirement.

## INTERNATIONAL RELATIONS

### Sierra Leone takes on the Soviet Union

#### London

THE Soviet Union is considering suing the Sierra Leone government for damages following the recent 8-day detention of the research vessel *Akademik Mstislav Keldysh* in the port city of Freetown.

The peculiar incident began last month when the oceanography research vessel, owned by the Academy of Sciences of the USSR, arrived in Freetown to pick up some representatives of a West German company that had recently completed repairs to the two deep-water submersibles carried on the ship. The plan was to test the repaired submersibles at sea 100 km west of Sierra Leone, where conditions are favourable at this time of year.

But when the ship docked at Freetown, it was boarded by Sierra Leone officials who removed the captain and several others, and questioned them in a local hotel. When the West Germans arrived in Freetown by air, they too were detained and questioned.

The Soviet foreign ministry immediately issued a strongly worded protest, calling the detention of the ship "a case of deliberate provocation". Soviet officials maintain that the Sierra Leone government was given 72 hours' notice of the ship's intention to dock at Freetown. Other countries, including Britain, whose scientists were also aboard the vessel, seemed less inclined to become involved in the dispute.

The Sierra Leone government has not explained why the ship was detained. According to the ship's captain, the Sierra Leone government evidently believed either that the ship was on a military mission, or that it was intending to dump toxic waste within Sierra Leone's territorial waters.

The ship was eventually permitted to leave, but the normally cordial diplomatic relations between the two countries are now strained.

Vera Rich

None of the five faculty members at Berkeley judged incompetent was close to retirement age, says Rader, who emphasizes that the policy is not age discriminatory. "The aim", he says, "is to deal with incompetence without reference to age."

Elizabeth Fader, of the American Association of Universities, says that concern over incompetence is rising at many universities, although most of the proposals for dealing with it have focused on incentives for early retirement, aimed at offsetting the ending of compulsory retirement ages. The National Academy of Sciences has begun a study, expected to be completed in two years, of the potential consequences for universities of the elimination of compulsory retirement.

Marcia Barinaga

## SOUTH AFRICA

### Kane-Berman wins reinstatement

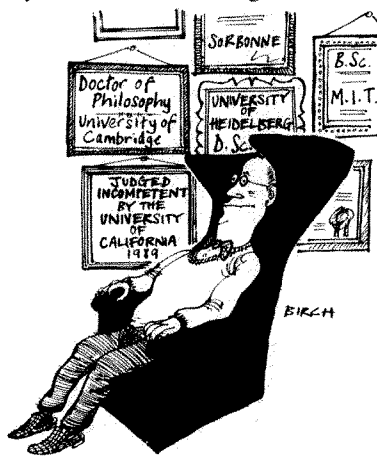
#### Cape Town

DR Jocelyn Kane-Berman, who was dismissed as superintendent of Groote Schuur Hospital in November after naming Nelson Mandela as her choice of premier in a newspaper interview (see *Nature* 338, 4; 1989), has been reinstated in her post with immediate effect.

The Administrator of the Cape Province, Mr Gene Louw (under whose aegis hospital administration falls) said on 10 March: "I can merely say that I took the initiative to bring it about. I thought it was in the best interests of Groote Schuur Hospital." This complete change in the government's attitude seems to be related to an appeal by Kane-Berman to have her reinstatement ordered by the courts. She had already served papers on Louw and other provincial officials, and her case was due to be heard in the Cape Supreme Court on 20 April. In December, Louw described Kane-Berman's removal as "final from the outset", and last month in parliament the Minister of Health and Population Development, Dr Willie van Niekerk, refused to intervene.

Kane-Berman has now agreed to withdraw the legal proceedings, and agreement has been reached on costs. She has, however, retracted her comments in the weekend newspaper in which she made her "controversial" statement, and has tendered her apology towards those persons and offices that were embarrassed. It is rumoured that South Africa's ailing State President, P.W. Botha personally ordered Kane-Berman's removal, although this has been denied by government spokesmen.

Kane-Berman, who described herself as "quite delighted" by her reinstatement, said: "I'm very pleased that the matter has been amicably resolved." **Michael Cherry**



statement is so vague as to be worthless, according to UC Berkeley engineering professor Ernest Kuh, chairman of the committee responsible for the new proposals. But the new policy, he says, breaks new ground, and other universities may wish to follow.

Under the new proposals, a faculty member, to be judged incompetent, must have both "ceased to engage in serious scholarship or creative activity for a substantial period of time" and be teaching in a manner "so inadequate that it is a dis-

# Kansai science city takes root

## Kyoto

WITH the opening last week of a new advanced telecommunications research institute, the Kansai region of Japan took its first step towards building a massive \$25,000 million 'Science City' designed to challenge the Tokyo region's dominance in high technology.

The Kansai region, encompassing Kyoto, Osaka and Kobe, is a powerful force in the world economy. Its gross 'national' product is twice that of Australia or the Netherlands, and by 1992 it will have Japan's first 24-hour international airport, providing direct access to foreign capitals.

Tax concessions will encourage research and educational institutes to move to the city. But the intention is to avoid the mistakes made in Japan's last giant science city project at Tsukuba, an hour's ride from Tokyo. Tsukuba was created by relocating 46 government research institutes and two universities from cramped quarters in Tokyo to a undeveloped site. But the city's cultural amenities never caught up with its laboratories and many of its research personnel prefer to commute from Tokyo than to live among the paddy fields.

Kansai's science city is planning for slower and more dispersed growth that will integrate it into surrounding communities. And rather than government laboratories, its focus will be on the development of "the basic sciences and their combination with applied technology". To help towards this objective, an International Institute of Advanced Studies, modelled on those at Princeton and Aspen in the United States, and a new national library, to rival that at the Diet in Tokyo, were planned as early components of the city. The institute will be built alongside

Advanced Telecommunications Research Institute International (better known as just ATR), the first major new laboratory at the city site.

ATR, backed by private industry, the Ministry of International Trade and Industry (MITI) and the Ministry of Posts and Telecommunications, sees little challenge left in developing telecommunications to move information economically from one fixed location to another. Rather, the institute looks to the long-term basic research that will provide automatic translation telephones, totally portable communications systems making it possible to call "anyone, anywhere, at any time", and neural-network computers to provide friendly man/machine interfaces. The institute has begun work with 160 researchers and is expected to have 300 by 1995.

Two government-supported projects are likely to come to the city: a MITI Institute for Ion Engineering, and a Ministry of Education, Culture and Science (MESC) graduate study institute. The MESC institute will be Japan's second 'graduate university', designed to boost the comparatively low number of postgraduate students in Japan, provide graduate students for the research institutes which are not attached to universities and exercise MESC's newly approved right to accept industrial funding for university chairs.

Private-sector companies will provide the great mass of the research institutes. Land has already been set aside for 10 such institutes. Biotechnology may turn out to be a strong theme, given that more than half of all Japan's major biotechnology and pharmaceutical companies are based in Kansai.

Alun Anderson

## MICROCHIPS

# Britain bows out of hi-tech

## London

THE sale last week of Britain's microchip company, Inmos, best-known for its revolutionary transputer, to SGS-Thomson (ST), a semiconductor manufacturer formed by the Italian and French governments in 1987, could mean that Europe has a semiconductor group capable of competing with the world-leaders from Japan and the United States. But the loss of British control illustrates the unwillingness of the country's industry and government to take a longterm view of investment in technology.

Britain's only other microchip manufacturer, Plessey, is also likely to go from national control into the hands of the West German company Siemens and the British company GEC; the takeover is under review by the Monopolies and Mergers

Commission. The British government, after launching Inmos in 1978, sold it six years later, before it made a profit, to the entertainments group Thorn EMI. But Inmos was seen as a high risk for the lighting and television rental group, making profits last year for only the first time since the beginning of the 1980s.

There were fears of redundancies in the three main Inmos plants, which employ over 1000 people, but a spokesman for the company says that ST plans to increase investment in the company, making Britain the headquarters of its microprocessing capability and possibly creating more jobs. For the sale of Inmos, Thorn EMI receives a 10 per cent shareholding in ST, and will continue to obtain the revenue from some patents on Inmos technology.

Christine McGourty

## Technology transfer

THE transfer of technology from universities and national laboratories into industry receives less government support in Britain than in West Germany, France and Japan, despite the relatively low industrial investment in research and development in Britain which makes technology transfer more important, according to a report published last week by Britain's National Economic Development Council. The report compares efforts at technology transfer in Britain, the United States, Japan, France and West Germany. Britain's efforts compare badly with those of its competitors. The report says that British companies are reluctant to take up external technology and do not view technology as a long-term asset.

C.McG.

## Radioactive waste

THE choice of sites for a deep repository for low-level and intermediate-level radioactive waste in Britain has been narrowed down to two: Sellafield in Cumbria and Dounreay in Caithness. Both are already the sites of nuclear installations. After permission is obtained from the planning authorities, geological investigations will determine which site is chosen. United Kingdom Nirex Limited, which is responsible for the disposal of low- and intermediate-level radioactive waste says each repository could cost about £3,000 million and should be in operation by 2005.

C.McG.

## Language schism

ANATOL Sarokin, a geologist with the Byelorussian Hydrogeological Survey, has been threatened with dismissal for insisting on his right to use the Byelorussian language for his field notes and reports. His superiors say that only Russian may be used at work, but Sarokin maintains that as it is a Byelorussian survey, reports should be in Byelorussian — particularly at a time when so much emphasis is being publicly put on the fact that the Byelorussian Republic is 'bilingual'. But the directors of the survey say that reports must be in Russian because a copy has to be sent to Moscow, and they cannot afford to have them translated. Sarokin's case has been taken up by the weekly *Litseratura i Mastastva*, the main advocate of *perestroika* in the Byelorussian Republic.

V.R.

## Greenpeace recognized

THE Baltic Marine Environmental Protection Commission, established in 1980 by the governments of the seven littoral states (Denmark, Sweden, Finland, the Soviet Union, Poland and East Germany and West Germany) has changed its statutes to give observer status to the non-governmental group Greenpeace. At its regular meeting in February, the commission also resolved to set up an early-warning system for incipient environmental disasters; each participating state will warn the others if it observes unusual and disquieting phenomena in the marine environment.

V.R.



## Canada's NASA

CANADA finally has a space agency. After months of squabbling over where it should be located, the government announced earlier this month that the new agency's headquarters would be in the greater Montreal area. Until now, responsibility for Canada's space activities fell to many ministries. The decision to base the new agency in the province of Quebec ends a political battle between predominantly French-speaking Quebec and the predominantly English-speaking Ontario that held up plans to establish the agency. In addition to coordinating Canadian activities on the space station, the agency will manage RADARSAT, a planned Earth remote-sensing satellite, MSAT, an advanced communications satellite, and the Canadian astronaut office. The current fiscal year's space budget is C\$120 million, rising to C\$150 million next year.

Larkin Kerwin has resigned as president of the Canadian National Research Council to become president-designate of the new agency. J.P.

## President removed

DR Pulat Khabibullaev, nuclear physicist and former president of the Uzbek Academy of Sciences, has been replaced as president of the Uzbek Republic on the grounds of corruption. He was accused of having "actively assisted" the scientific careers of close relatives of now-disgraced local party leaders, procuring them posts for which they were insufficiently qualified and which they treated as virtual sinecures. Furthermore, he is said to have used his position as vice-president and then president of the academy to have his name attached as co-author to no fewer than 322 scientific papers to which he had contributed nothing. Called to account before the bureau of the Central Committee of the Communist Party of Uzbekistan, Khabibullaev made a formal self-criticism, whereupon the Party recommended that he be allowed to resign as president of Uzbekistan and "transferred to scientific work". V.R.

## Fang speaks out

DR Fang Lizhi, the Chinese astrophysicist and dissident who was prevented by the police from attending US President George Bush's banquet in Beijing, has not let the incident pass unchallenged. At a press conference at his home the following day, he suggested that the reluctance of the Chinese leadership to have dinner with one dissident on one single occasion must surely raise doubts about their "promised tolerance" of the future presence of non-socialist Hong Kong in post-1997 China. Official Chinese spokespersons continue to attack the original invitation as "disrespectful" to the president's official hosts — the Chinese leaders — and US reaction to Fang's exclusion as "irresponsible". V.R.

# Outside influences resented

## São Paulo

SOUTH American government officials promised at a series of meetings last week to respect the rain-forest environment while promoting development, and called for more research on the damage to rain forests. But a concurrent scientific meeting on rain-forest ecology dismissed these promises as mere rhetoric, and complained that researchers are being denied resources.

The foreign ministers of Ecuador, Brazil, Colombia, Venezuela, Peru, Surinam, Guiana and Bolivia gathered in Quito, Ecuador, last week for the third meeting of the Amazonian Cooperation Treaty, signed in 1978 to defend the region's environment. The ministers favour "rational" use of Amazonian resources, and condemn "any foreign interference on the policies and actions that the Amazonian countries make in the region".

The governors of several Brazilian states were in Manaus to discuss the newest federal environmental protection programme called "Nossa Natureza" ("Our Nature"). Announced last year as a response to growing internal and international criticism of rain-forest devastation, the programme consists of legislation strengthening forestry and mining codes and creating new protected areas. Brazilian President Jose Sarney is due to sign these measures on 6 April.

But scientists from INPA — Instituto Nacional de Pesquisas da Amazonia (National Institute of Amazon Research) — think otherwise. They feel left out of the planning of "Our Nature" and fear that it is going to be just another meaningless declaration of good intentions. Researchers from the institute held a last-minute "alternative" meeting on 7 and 8 March in Manaus to draft a response to the "Our Nature" approach, but were prevented from presenting their conclusions to the governors meeting nearby.

Brazilian Minister for Industrial Development, Science and Technology, Roberto Cardoso Alves, said in February that the rain forest is "practically intact", with just 1 per cent destroyed. Estimates from INPA put the figure at 8 per cent.

If governments are resisting foreign intervention, the same cannot be said for researchers. INPA has many foreign scientists, mainly US and German. One US scientist, Philip M. Fearnside, has become the institute's media star, by his constant advocacy of preservationist measures. He complains that red tape is drawing away many researchers from the Brazilian side of Amazonia: there are stories that some foreign scientists have had to wait a year for a visa to visit Brazil.

INPA researcher Muriel Saragoussi says the institute may lose more than a

third of its 284 researchers if a government decision to dismiss all public servants with less than five years on the job takes effect. The proposed layoffs are a result of a January economic plan by the federal government to try to stave off hyperinflation. Another consequence of this plan is a reduction of 50 per cent in the financing of research institutes.

Research on rain forests will certainly be an important part of the planned International Geosphere-Biosphere Programme (IGBP). The programme's executive director, Thomas Rosswall, toured last week through several Latin American countries to promote the programme. He diplomatically stated in São Paulo that Brazil has sovereignty to decide what to do with its natural resources, but added that all countries will have to take responsibility for what happens to the world's climate. He compared the situation of the rain forest with the emission of sulphur dioxide by Britain; only when scientists showed that British emissions were polluting Europe did the British government take measures to curb them. Brazil may face similar pressure when the true importance of the rain forest to global climate change is conclusively shown. IGBP is likely to place one of its proposed global observatories in the Latin American rain forest. Ricardo Bonalume Neto

## Mercurial approach

### São Paulo

THE Brazilian government has been somewhat mercurial in its approach to science and science policy. Two months after deciding to abolish the Ministry for Science and Technology, it announced last week that it was creating a new, cabinet-level Special Secretary for Science and Technology. The two-month-old Ministry of Industrial Development, Science and Technology, which emerged in the wake of the departure of the four-year-old science ministry, now becomes the Ministry for the Development of Industry and Commerce.

Critics say this confusion of names and ministries underlines the lack of a coherent policy for Brazil's scientific development. Nevertheless, the Brazilian Society for the Advancement of Science praised the creation of the new secretaryship. The society lobbied intensely to recreate the ministry in order to have a single entity to coordinate science policy.

Financial problems for science persist. Some ministry institutes now in the new secretary have less than half the money they need to function. Researchers at the National Institute of Amazon Research (INPA) have to buy fuel for their jeeps out of their own pockets. Ricardo Bonalume Neto

## UK UNIVERSITIES

## AUT exam boycott enters week 11

London

THE end of the 10-week-old lecturers' boycott of British university examinations is no closer after weeks of intense negotiations between the Association of University Teachers (AUT), the Committee of Vice-Chancellors and Principals (CVCP) and the government. If the action continues, final year students may not be awarded classified degrees, and universities fear students may take legal action against them. Already mid-term examinations have been cancelled and summer papers are not being prepared.

The CVCP is meeting this week to discuss how to minimize the effects of the strike on students. Vice-chancellors could decide to award unclassified degrees based on tutors' reports or to ask students to sit examinations after the summer, at the time when re-sits are usually taken. Meanwhile, they are hoping that AUT members will disagree with the council, the policy-making body of the union, which last week rejected the CVCP's latest offer of a pay rise. Members will be balloted in April on whether to accept the council's decision.

The offer of an increase of 6.5 per cent for 1989-90 was made after the universities received an undisclosed sum from the government, on condition that the strike is ended. The CVCP had asked for £187 million but received "a lot less than that", according to a spokesman. The union is certain that the universities can finance a larger pay increase, though Sir Mark Richmond, chairman of the CVCP, denies this, saying that the latest offer is final, and that to offer any more would bankrupt some universities.

Christine McGourty

## THINK-TANK

## Berlin academy fights closure threats

Munich

THE new left-wing government in West Berlin plans to close the 'Academy of Sciences and Technology in Berlin'. The academy was founded in 1987 by the then-conservative government as an interdisciplinary think-tank especially concerned with problems of technology and society. The Greens and Social Democrats now in power denounced the academy as an "old men's debating club" (see *Nature* 329, 659; 1987). The academy's research fields include solar energy, ageing and society, and the impact on German science of the exodus of researchers from Berlin in 1933. It had a 1988 budget of DM8 million, mostly provided by the West Berlin government.

The academy will fight for its existence. Spokesman Eberhard Vogt said that the "last word" had not yet been spoken about the future of the academy.

Steven Dickman

## DEFENCE RESEARCH

## Independent agency for UK

London

BRITAIN'S main defence research institutes are to be grouped together in an independent defence research agency in order to loosen their ties with the Ministry of Defence, their main customer.

At first the agency will be made up of four institutes: the Admiralty Research Establishment, the Royal Aerospace Establishment, the Royal Armament Research and Development Establishment and the Royal Signals and Radar Establishment.

The Aeroplane and Armament Experimental Establishment is also being considered, but the Chemical Defence Establishment (CDE) at Porton Down will not be part of the agency. A review carried out last year by a study team for the Ministry of Defence (MoD) said that, because of the political and public sensitivity about the work carried out there, and because of the role of the establishment in providing support to government on international arms control, the CDE should be excluded from the agency.

The agency will be expected to increase the work at the institutes carried out for industry, and will have to compete with industry for some contracts for the MoD. There is also likely to be a reduction in staff and in the number of sites. More than

12,000 staff work in the institutes at over 100 sites, most of which are in South-East England where land sales would be remunerative.

There are still many uncertainties surrounding the form of the new agency. A team has been set up to plan in detail the changes before the agency is formed in 1991. It could take the form of a trading fund within or outside the civil service, or it could be a government-owned public limited company. Full privatization has been ruled out.

Manpower is one of the important problems to be tackled. A serious shortfall in recruitment of young graduates, especially in electronics, computing and mathematics, combined with a high rate of resignations among the youngest and brightest of researchers, has led to a shortage of high-calibre staff. The defence research study team said that this could be solved by the introduction of more short-term contracts and by giving the agency more independence to fix pay scales, now set at civil servant rates.

The study team also stressed that in a new agency mechanisms must be put in place to safeguard strategic research, which could be neglected in the commercial climate.

Christine McGourty

## US SALARIES

## Less pay means empty jobs

Atlanta

THE AIDS epidemic may have helped the budget of the US Centers for Disease Control (CDC) to multiply to more than \$1,000 million a year in the past six years, but the hefty increase has not meant salary rises for its researchers.

Instead, the agency, the front-line of defence against epidemics in the United States which also helps to staunch outbreaks of disease around the world, is suffering from the same problems as the rest of the US government's research enterprise — those of hiring the best and the brightest talent.

Earlier this year, CDC's largest research centre, the 1,000-staff Center for Infectious Diseases, was forced to cast its net outside the United States to fill one of its top posts. Fred Murphy, director of the centre, made the unusual move of hiring one of Britain's top virologists, Brian Mahy, away from his directorship of the Agriculture and Food Research Council's Pirbright Laboratory. Five US scientists offered the job had turned it down because the \$75,000 salary was too low.

Mahy says he was not actively looking for a job when Murphy came knocking on his door, but recent shake-ups in the organization of British veterinary science (see

*Nature* 338, 191; 1989) made moving to the United States more attractive. He says he considered the CDC post an advancement and it offered him a 25 per cent raise in salary. Several other Pirbright scientists left with Mahy to work at CDC.

CDC's Murphy says he spends much of his time looking for prospective employees, and that it takes 6-12 months to find the right people for executive-level positions within his office and as directors for his centre's 12 divisions. He has had to fill seven "very senior positions" within the past year and half.

The most pressing vacancy at CDC is a replacement for CDC director James O. Mason, who has been chosen by the Bush administration to be the US Assistant Secretary of Health, in charge of the Public Health Service which oversees both CDC and the National Institutes of Health. While Mason or the new Secretary of Health and Human Services, Louis Sullivan, could turn the CDC directorship into a political appointment, George Hardy, acting deputy director of CDC, predicts that the job will be filled by a search committee, a process which could take several months. Mason is sure to take an active interest in selecting the new CDC director.

Carol Ezzell

# Fears of drought assuaged

## Berkeley & Boston

A MODERATELY wet winter has eased fears of a second year of drought in much of the mid-west and Rocky mountain areas of the United States, and long-range forecasts have even raised hopes of a year of bumper crops. But some regions still face shortages, and despite recent rains, rationing is already being implemented.

Last season's mid-western drought was probably a one-time occurrence, possibly caused by temperature anomalies in the tropical east Pacific. While some areas of the mid-west remain drier than normal, another extensive grain-belt drought is unlikely this year.

Californians now face an unprecedented third consecutive year of drought, as weather patterns in the Pacific diverted the jet stream and its storms away from the state for much of the season. Rainfall for most of the winter was less than half of normal, leaving the state in a dangerously dry condition, according to William Helms of the state drought centre. A wet March has provided some relief in the central part of the state, and the cities of Southern California were spared the full effects of the drought, but much of the San Francisco Bay area is still in crisis and beginning to bargain for water from other parts of the state.

Many Californians assumed the drought could not persist for three years in a row, as it never has in the 120 years during which records have been kept. So the current crisis, with its accompanying water rationing, came as a rude shock. Santa Clara County, home to California's semiconductor industry, is one of the hardest hit areas in the state. Dependent on local water supplies, the county has been little helped by the March rains, which fell mostly to the north and in the mountains to the east.

Reservoirs that feed Santa Clara's underground aquifer were empty in February for the first time in history, sending county officials begging for water loans from other parts of the state, amid fears that land in Santa Clara would begin to sink if more water were drawn from underground.

March rains have eased the plight of California's \$15,000 million agriculture industry, which earlier this season was warned of likely cutbacks in water allotments of up to 60 per cent. The State Department of Water Resources says cutbacks are still likely, but will be smaller.

In the north-east, the unseasonably warm and dry winter has also prompted concern over the prospect of severe water shortages. Water levels in key reservoirs in New York, Massachusetts and several other states are at or near historic low points, after four consecutive years of

abnormally arid weather in the region.

Last week, New York's Drought Management Task Force voted unanimously to recommend that a water emergency be declared for New York City and 11 other counties in the state. The move followed a declaration of water emergency last month in Massachusetts, giving the state government jurisdiction to enforce mandatory restrictions on water usage by municipalities.

Although the situation is most severe in Massachusetts and southern New York, representatives from the United States Geological Survey (USGS) and other state environmental agencies say that all the northeastern states in the United States have been affected by particularly dry conditions over the past year and

many areas in the region are threatened by drought.

In Massachusetts, the declaration of a water emergency came after reports from the Water Resources Authority that the state's largest reservoir, serving more than 40 municipalities, had dropped to its lowest level in 15 years. Similarly, reservoir levels in New York stand at only 55.9 per cent of their capacity, more than 30 per cent below the average for this time of year. Because Massachusetts and New York have had almost no snow this year, state water officials are concerned that the spring thaw will not provide nearly enough water to replenish the system.

The problem in Massachusetts is intensified further by water contamination at some local sources which has forced several municipalities in the state to close their local water supplies.

**Marcia Barinaga & Seth Shulman**

## SOVIET PSYCHIATRY

# New independent association

## London

NINETEEN Soviet psychiatrists, from Moscow, Leningrad and other major cities, have established the Independent Psychiatric Association (IPA) of the USSR. According to Dr Viktor Lanovoi, president of the new organization, the IPA does not intend to enter into conflict with the existing All-Union Society of Neuropathologists and Psychiatrists. Nevertheless, its founding statement makes it clear that it has come into being in response to the abuses that have compromised Soviet psychiatry in the eyes of the world.

Its founding statement declares that the IPA is open to "all those who consider it necessary . . . to defend the doctor against social and political pressure, and to defend people, healthy or ill, against extreme socio-political and psychiatric arbitrariness".

The IPA founding follows hard on the heels of a two-week visit to the Soviet Union by a team of US psychiatrists to investigate charges of psychiatric abuse. International criticism of Soviet psychiatric practices resulted in a decision in 1983 by the All-Union Society of Neuropathologists and Psychiatrists to resign from the World Psychiatric Association (WPA) to forestall its impending expulsion. The society has now filed an application for readmission, but the IPA has announced its intention to apply to the world body as well, and only one national society in each country can be a member of the WPA.

The US visiting delegation was allowed into the Soviet Union under conditions similar to those governing arms-control inspectors. It visited hospitals and patients of its choice. IPA member Aleksandr Podrabinek, who some 10 years ago

became the first person within the Soviet Union to attempt to monitor psychiatric abuse, worked closely with the delegation. Podrabinek maintains that recent, much publicized "improvements" in the Soviet treatment of penal psychiatric cases were largely cosmetic. Moreover, rather than releasing all political "patients", Podrabinek points out, the authorities have actually hospitalized some new ones.

Nevertheless, Podrabinek welcomed the US visit as an important breakthrough. Now back in the United States, the visiting team will issue an executive summary of its findings in one month. The American Psychiatric Association, one of the trip's organizers, has taken no position on the All-Union Society's application for readmission to the WPA, and will not comment until the report is produced. But Podrabinek has issued his own account. He stressed the shock felt by many of the Americans at the lack of compassion with which the Soviet psychiatrists treated their political "patients".

Although the Soviet psychiatric establishment clearly needs to win over world opinion, and in particular that of the powerful US vote in the WPA, official opinions, according to Podrabinek, were divided as to whether the proposed US visit would do them more good than harm. Up to the last minute, he said, the Soviet side kept altering the terms of the visit — deciding, for example, that the Soviet psychiatrists and not the patients nor the Americans would select which relatives or friends would accompany the patients during the examinations. In the end, the patients simply brought the relatives of their choice, and the Soviet side made no attempt to challenge these choices.

**Vera Rich**



## NUCLEAR POWER

# Holy river no obstacle

New Delhi

IGNORING protests from environmental groups, India last week commissioned the controversial nuclear power reactor at Narora on the banks of the sacred Ganges in the state of Uttar Pradesh, 140 km east of Delhi.

The 230-MW reactor is the first of two units at India's fourth nuclear power station. Commissioning of the second unit at Narora has been put off until 1990 because of a lack of indigenous heavy water.

The twin reactors of the \$500-million Narora Atomic Power Station (NAPS) are based on the Canadian CANDU design that uses natural uranium fuel and heavy water as moderator and coolant. After Canada severed nuclear ties in 1974, India on its own built two CANDU at Kalpakkam near Madras in 1985. NAPS is the second totally indigenous station and, like the Madras reactors, is outside the control of the International Atomic Energy Agency (IAEA). The two US-built boiling-water reactors in Tarapur near Bombay and the two reactors near Kota in Rajasthan, built by Canada, are under IAEA inspection.

The siting of the new power station at Narora has been the subject of a bitter controversy between the Department of Atomic Energy (DAE) and environmental groups such as the Committee for Sane Nuclear Policy (Cosnup). While the Tarapur and Madras stations are sited near the sea, and the Kota reactors use cooling water from a lake, NAPS is the first Indian nuclear plant to be built near a major river. Although only make-up water will be drawn from the Ganges for the closed circuit cooling system, treated effluents will drain into the river, which is currently being cleaned up under a \$250-million 'Ganga action plan'.

Environmental groups have organized a 'Save Narora' campaign that is concerned about possible radioactive pollution of the Ganges, the life-line for 200 million Indians living in its basin. The new reactor at Narora is also the first reactor in India to be located in a seismic zone. Critics say the risk of radioactive pollution of the Ganges is heightened by the fact that the reactors are standing on alluvial soil only 50 km away from the Moradabad Fault which was the centre of an earthquake in 1956.

These fears have been dismissed by DAE, which says the reactors have double containment systems and have been designed for automatic safe shutdown in case of earth tremors. The effluent treatment will be such that there will be 'zero-radiation discharge' into the Ganges. Critics, unconvinced, note that the extra money spent on making the plant earth-

quake-proof could have been saved by siting the plant elsewhere. Earthquake-proof design considerations were mainly responsible for cost and time overruns. NAPS took 12 instead of 7 years to complete and its cost had doubled.

Ironically, commissioning of the new reactor at Narora will not add to India's installed nuclear power capacity but only bring it to the level that existed in 1986. Since then, output from the Tarapur reactors has been deliberately reduced to lessen the radiation dose to personnel there, and one of the two Kota units has been shut down due to a leak from the end plate of the reactor vessel. Attempts to plug the leak permanently have so far failed.

By the year 2000, India plans to produce 10,000 MW or 10 per cent of total electricity demand from nuclear reactors. This would require commissioning of two or three reactors per year, an impossible task. Even if DAE could mass-produce reactors, it has to face a growing anti-nuclear lobby.

Opposition is already mounting against the nuclear station under construction at Kaiga, in the midst of rain forest in western Ghats, and the two 1,000-MW reactors proposed to be built by the Soviet Union at Kodangulam on east coast of south India.

K. S. Jayaraman

## Soviet citizens not impressed by IAEA

London

PLANS for a nuclear power and heating station in Gor'kii in the Soviet Union have roused considerable opposition from local inhabitants, who, in the aftermath of the Chernobyl accident, are concerned at the proposed siting of such a station only a few kilometres from the city centre. The Soviet nuclear planners have now agreed to call in experts from the International Atomic Energy Agency (IAEA) in Vienna, to assess the safety aspects. If the IAEA gives its approval, the station could begin supplying the city with heat next summer.

Twelve such stations were originally planned for the European part of the Soviet Union, with Gor'kii as the prototype. But the partly built station at Minsk is already being modified to operate on conventional fossil fuel, under local pressure. And Soviet public opinion, it seems, is not always ready to accept the IAEA experts as disinterested parties. Last autumn, when Dr Murray Rosen of IAEA tried to assure local people that the RBMK (Chernobyl-type) power station at Ignalina posed no threat, he received boos and catcalls.

Vera Rich

## CRAFOORD PRIZE

## Pioneer in rocketry and radiation discovery

Washington

THIS year's Crafoord Prize has been awarded by the Royal Swedish Academy of Sciences to James Van Allen of the University of Iowa.

The prize, worth about \$250,000, is given annually in an area not covered by the Nobel prizes. This year's recipient is cited for his work in magnetospheric physics, his best-known accomplishment being the discovery in 1958 of the 'Van Allen' belts, regions in the upper atmosphere in which charged particles of solar origin are trapped by the Earth's magnetic field.



Van Allen began his scientific career as an experimental nuclear physicist and became a pioneer of scientific rocketry, designing both novel detectors and the rockets that carried them. The Aerobee rocket was his work, and he was chief scientist for the Explorer I satellite, which found the radiation belts that carry his name. At the University of Iowa in the early 1960s, he and his pupils designed and built an important series of satellites.

These days, Van Allen is known as a critic of manned space exploration, believing it to be a scientifically unproductive extravagance.

David Lindley

## FOSSIL LOSS

## Tourism falls victim to 'Tyrannosaurus'

Tokyo

JAPAN's only fossil to have acquired the exalted status of a national 'natural treasure' was toppled from its perch last week when word finally reached the press that the carnivorous *Tyrannosaurus* dinosaur discovered in 1976 at Mikasa City, Hokkaido, is actually a lizard, *Mosasaurus*.

Doubts about the fossil's identity have been circulating privately in the Japanese geological community for some time. Now that the truth has come out from the National Science Museum's Ikuo Obata, who made the original misidentification, Mikasa City (population 20,000) is stuck with a massive concrete replica of an erect *Tyrannosaurus*, a thriving industry supplying *Tyrannosaurus* souvenirs — from key rings to sweets and pickles — and a museum containing the fossilized head of what, after all, is a *Mosasaurus*.

City officials are deeply disturbed, saying that the *Tyrannosaurus* business is all they had to stop the outflow of people to big cities. The Cultural Agency, which granted the 'natural treasure' title says it is "studying the situation".

Alun Anderson

## Reprints still in demand

SIR—Following on the discussion on reprints (*Nature* 336, 708; 1988) I should like to raise two points that very much concern a minority of workers like myself.

First, as a full-time school teacher who has a reasonable output of papers and whose place of employment is not in a university or professional laboratory, I rely on reprints to keep me abreast of my subject. To start with, I rely on a small nucleus of workers who automatically send me reprints of relevant papers. From those, I can then follow up what seem to be useful references by sending for more reprints. I do not have access to abstracting systems nor to libraries that stock journals except on the rare occasions when I make the journey to London.

Second, as an isolated entomologist, I become aware of others with an interest in my field only by receiving reprint requests.

I would be very sad indeed if Ivor Smith's attitude started a widespread movement away from the reprint system.

J.T.C. SELICK

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SIR—Ivor Smith expresses his discontent about the reprint requests he received for his two-page paper, all of which he ignored. He claims that "the European reprint is a disappearing commodity". I believe this attitude is detrimental to at least one part of the scientific community and is inconsistent with efforts to improve cooperation and exchange of information between scientists in the West and East (*Nature* 337, 1; 1989).

I have recently received 153 requests for a one-page paper, 90 of them from countries where neither photocopiers nor scientific journals are abundant. I myself worked for five years in an institute of the Czechoslovak Academy of Sciences. The institute was well equipped and financed and theoretically one could get any paper, but it could take weeks or months, depending on the availability of the journal. It was not possible to order all the papers one wanted that were not available in the library — the capacity of the service would have been far exceeded. Bureaucracy and censorship made things even more complicated: for example, issues of *Nature* were sometimes incomplete. One had to order a copy of a specified article and sign a declaration that the copy would be used only for scientific purposes. Such measures will vanish with the general political shift, but the availability of the scientific literature will not improve significantly in the near future. Reprints remain an important source of information for researchers in the Eastern bloc.

Ivor Smith uses the difference between the cost of postage and that of a photocopy as an argument against reprints. Such reasoning is inappropriate for scientists in the Eastern bloc: as money for postage is limited, some workers pay for this service themselves. This is necessary especially in fast-evolving fields or where more than one field has to be pursued simultaneously.

The decision whether or not to answer a request is a personal one. It depends also on postal funds available. Nevertheless, there is a consensus in the scientific community that reprint requests should be answered. Not to do so is unfair to those who conform — journals that provide authors with free reprints and researchers who ask for them. I suggest, therefore, that authors who cannot or do not intend to send out reprints make an arrangement to spare others the unnecessary costs: as a part of the corresponding address (in order to be included in indexing periodicals such as *Current Contents*), a note "no reprints" should be inserted. This could also serve as a hint for the journals in question to save the costs of producing free reprints.

PETR KARLOVSKY

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SIR—If one wants a copy of a recent paper that has photographs showing ultrastructural detail (common in microscopy and biology journals), then a photocopy is usually not good enough.

PATRICIA A. MOSS

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## Sadly confused

SIR—Frank W. Dobbs' criticism (*Nature* 337, 497; 1989) is sadly correct, as you noted, but he is sadly mistaken on who was confused: it was Kepler who analysed Tycho's measurements and not Copernicus who analysed Kepler's — to wit: Copernicus 1473–1543, Tycho 1546–1601, Kepler 1571–1630.

KLAUS SANDER

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

## African birds

SIR—Tim Crowe's diatribe (*Nature* 338, 11–12; 1989) on the seventh Pan-African Ornithological Congress (PAOC) left me wondering if we had actually attended the same event. I did not think I was alone in finding it a highly enjoyable, well-organized and scientifically stimulating conference which could also (for the first time, it seems) honestly be described as 'pan-African' in its representation.

It is possible to understand Crowe's frustration over the processing of his and his colleagues' visas. But his obsession with the so-called 'exclusion' of South Africans prevents him from grasping the fact that the future of African ornithology depends on our building a basis for it over the entire continent. In this regard, his dismissal of the papers given by African delegates is particularly unhelpful (not to say offensive). Unfortunately, few African ornithologists will have had access to resources and training of the calibre provided by a FitzPatrick Institute. This makes it all the more important that young scientists have the chance to attend international conferences such as this one, where they can present work, receive constructive criticism and learn from the example of their more experienced colleagues. Indeed, this is one of the best reasons for the congress's new regionalism. The fact that last year's meeting was held in East Africa allowed many scientists to attend who would otherwise never have had the chance, and at future congresses this opportunity will be extended to those in Central and West Africa as well.

Crowe's nostalgia for the days when the PAOC was run by South Africans for South Africans is profoundly unrealistic. I doubt that the absence of many distinguished South African ornithologists can have cheered any delegate to the seventh congress, even among the supposedly "virulent anti-South African elements". But this situation clearly arose as a result of the distortions induced by apartheid, distortions which, while they act to impoverish African science, cannot simply be ignored. The cost of guaranteeing South African participation in all future congresses would be the continued paralysis of pan-African ornithology, and the seventh PAOC decided that this cost is too great. Eventually, the isolation of South African ornithologists must be ended through political change. In preparation, Crowe's colleagues would do well to follow the positive steps outlined at the end of his article — in particular to remedy the disgraceful lack of black South Africans among their number.

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# Novel materials from theory

Marvin L. Cohen

The structures of crystals can be predicted using information about their chemical composition as the only input. Such approaches will greatly aid the search for new materials.

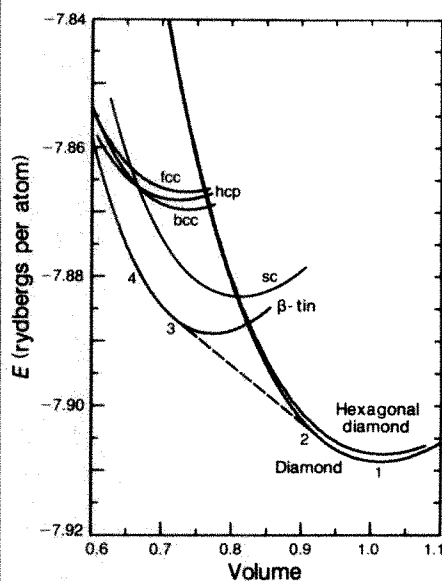
THE "continuing scandal" referred to by John Maddox in his recent article "Crystals from First Principles"<sup>1</sup> is "that it remains in general impossible to predict the structure of even the simplest crystalline solids from a knowledge of their chemical composition". If the words "in general" are intended to mean for all cases of simple crystalline solids, then the statement is certainly true. But for some cases, the implied goal has already been achieved; some of the approaches used strongly resemble what Maddox feels "ought to be possible".

Silicon is probably the best example. At atmospheric pressure and at room temperature, silicon is a tetrahedrally coordinated semiconductor with the diamond structure. In 1980, M.T. Yin and I showed that starting with the atomic number of silicon, 14, a precise estimate of the total energy of solids can be generated composed of specific arrangements of silicon cores of charge +4 embedded in a sea of itinerant valence electrons<sup>2</sup>. A core contains the silicon nucleus and ten tightly held 'core' electrons, which are assumed to be unchanged in going from the atomic to solid state. The four valence electrons from each atom are free to wander throughout the crystal and are expected to arrange themselves in a manner that achieves a minimum energy state. So, for an assumed crystal structure, the total energy of the core and valence electrons could be computed as a function of the core separation, or volume per unit cell.

The next step is that anticipated for future calculations by Maddox. A list of good candidate structures is used to choose various arrangements of the cores, and their energy is compared for many assumed volumes. In the original study<sup>2</sup> on silicon, seven structural phases are examined (see figure). These are diamond, hexagonal diamond, white tin ( $\beta$ -tin), simple cubic (sc), body-centred cubic (bcc), hexagonal close-packed (hcp) and face-centred cubic (fcc). Several more structures have been considered since 1980. As shown in the figure, the diamond structure was computed to have the lowest energy among these for the volume appropriate for atmospheric pressure. The minimum of the curve of energy versus volume for the diamond structure gives the lattice constant and its curvature yields the compressibility or bulk modulus. The calculated results agreed with experiment to within 0.4 per cent for the lattice

constant and 1 per cent for the compressibility, which is impressive considering that the input is only the atomic number and the list of candidate structures.

This calculation gives a starting point for Maddox's goal that, by the use of a powerful computer, a chemical formula is typed in to "obtain, as output, the atomic coordinates of the atoms in a unit cell". In fact, for silicon even more has been done.



Total energy curves of seven structural phases of silicon as a function of volume normalized to the volume of the diamond phase. The dashed line, which is the common tangent between the diamond and  $\beta$ -tin phase, represents the path for this pressure-induced structural transition. Its slope gives an estimate of the transition pressure. (From ref. 2; see text for further details.)

When the atomic mass was added to the input information, the vibrational spectrum, the electron-lattice interactions, anharmonic lattice couplings, cohesive energy and other properties, together with their pressure dependence, were obtained. But most of these properties were known beforehand. Despite the first-principles nature of the calculation, its success does not completely meet Maddox's challenge — "the goal of calculating from first principles the crystal structure of a compound of which nothing is known except chemical composition".

But examination of the work on silicon again shows that, to a large extent, even this goal has also been achieved. Although the calculation described above demonstrates that diamond is the structure of

lowest energy at atmospheric pressure, its energy increases above that of some other structures as the volume per atom is reduced, which can be achieved in the laboratory by subjecting a sample to pressure. The reduced-volume calculation showed that silicon should be metallic and stable in the white-tin structure at pressures around 100 kilobars and in the hexagonal close-packed structure at 400 kilobars. The path for the diamond to white-tin pressure-induced transformation is shown by the dashed line in the figure. This line is the common tangent between the curves, and the points labelled 1, 2, 3 and 4 represent volumes at which the solid (1) is the diamond structure, (2) is beginning the transition, (3) is ending the transition and (4) is in the white-tin structure. The calculated values of the transition volumes at (2) and (3) are within 2 per cent of their measured values. Using the slope of the common tangent, the transition pressure for the transformation was calculated and it is within several per cent of the measured values. The white-tin transition was known, but the hexagonal close-packed structure was a mere prediction. Experiments were done, and the predicted structure was found at the estimated pressure. The lattice constant and many other properties were also predicted successfully. Another simpler hexagonal structure appeared at lower pressures, and the theory was used to compute its properties.

When the calculations were extended to calculate the lattice vibrational properties and the interactions of the valence electrons with the vibrating core, there was enough information to predict that simple hexagonal silicon should be superconducting at temperatures in the 5–10-kelvin range and that hexagonal close-packed silicon would superconduct around 4–5 kelvin. The predictions were verified<sup>3,4</sup>, as was the pressure dependence of the transition temperature.

So there is an example of a successful prediction of the existence of high-pressure structural phases, their lattice constants, electronic and lattice properties, and even superconductivity. The calculation proceeds from first principles and requires only the atomic numbers, atomic masses and candidate structures as input. Silicon is the prototype material, but many systems have been studied with similar success. For example, the simple hexagonal structure of germanium was success-



fully predicted to exist in the 800-kilobar range. Other materials that have been investigated include metals, III-V semiconductors and some ionic insulators. Maddox discusses graphite, diamond and ice; the first two have been analysed successfully, but the last has not.

How do these calculations proceed, and what is the state of the art? Maddox focuses on a study<sup>5</sup> of polymorphs of silica, an interesting calculation which requires input information about  $\text{SiO}_4^{4-}$  clusters. Maddox describes the achievements of the calculations and mentions some reservations about them. He notes the need to add fictitious positive charges and to set the distances between oxygen atoms and the point charges equal to the observed Si-O distance, for example, observing that it is wrong to say that "everything depends on what the programmers have done". Most researchers in this area would agree with this observation, but there are approaches which do not call for caveats of this kind.

The present state of this area can be assessed by a more detailed examination of the silicon calculation<sup>2</sup>. The procedure is to compute the total energy by summing the interactions between and among the cores and valence electrons. The core-core repulsion is computed with standard techniques for evaluating the Coulomb electrostatic energy between fixed cores using Madelung sums (the sum of Coulomb repulsions over a whole lattice). The electron-electron contribution to the energy can be estimated directly from the electron density as a function of position in the crystal. The electron-core term is more difficult because the interaction between a valence electron and a core has two main components: the attractive Coulomb interaction and the repulsion arising from the Pauli exclusion principle. Both of these can be incorporated into a pseudopotential which can be constructed for all the atoms in the periodic table and beyond. These potentials are particularly suited to the description of electrons with low angular momenta, but that is not a severe limitation. In principle, solids with many atoms in a unit cell can be studied.

### Refined approaches

The progress in this area is well-documented in the literature (see, for example refs 6,7) and many researchers are contributing to the refinement and improvement of these approaches. There is particular interest in the development of schemes to treat excited electron states, to improve the precision of calculating static properties such as cohesive energy and to avoid the need of starting with a list of candidate structures.

There has been significant progress in all three areas. It is now possible to use the first-principles approach to compute optical and photoemission properties of

solids without any experimental input. When electrons are excited by light or heat they interact differently with the other electrons in the solid. Several computations of these changes have been done based on new theoretical approaches<sup>8</sup> and fairly complex calculational techniques. The refinements in the evaluation of electron-electron interactions beyond schemes which use only the position-dependent density of the electrons are mostly based on extensions of a statistical approach to the encounters between a finite number of electrons. These quantum Monte Carlo simulations<sup>9</sup> provide the data needed to treat the electron-electron interactions more exactly.

Finally, statistical approaches<sup>10</sup> may allow the determination of crystal structures without tests of candidate structures. By allowing cores to move randomly and in a manner so as to minimize the energy of the entire system, including contributions from the sea of valence electrons, it is expected that the lowest energy structure at a given volume will be the same as nature's choice for that volume. There are problems with this approach, which arise because systems tend to choose local energy minima that are configurations of low energy with core positions close to the starting configuration. These may not be the lowest or global energy minimum, and the solids they predict may be unattainable metastable states of the system. Nevertheless, this problem should be overcome before too long.

In general, the outlook is bright for the development of theoretical approaches with the predictive power envisaged by Maddox. In addition to enabling us to simulate extreme conditions for making materials, with theoretical models we can easily vary the constituent elements in a compound. Unlike experiment, where each change of an element usually requires starting the synthesis all over again, theoretical modelling requires small adjustments and new combinations are tested quickly. The search for new materials using theory can be made more efficient and faster if partially empirical approaches are used. For example, a calculation of the bulk modulus ( $B$ ) or its inverse, compressibility, for a class of tetrahedral semiconductors requires significant computer time when the first-principles theory is used. But a simpler scaling theory<sup>11</sup> gives the results with comparable accuracy using a hand calculator. The formula is

$$B = (1.971 - 220\lambda)d^{-3.5}$$

By inputting  $d$ , the bond length in Ångströms, and the ionicity parameter  $\lambda = 0, 1, 2$  for Group IV, III-V or II-VI semiconductors, respectively, the bulk modulus  $B$  in gigapascals is obtained with remarkable accuracy<sup>11</sup>. The full theory was used to provide the basis and the tests of this simpler approach, but now the

formula for  $B$  can be used to give insight into materials development.

### Hard as diamond

An example of the use of the formula for  $B$  is in the search for materials with hardness comparable with that of diamond. Hardness is generally directly related to the bulk modulus, and diamond has the largest bulk modulus of all materials. It is a tetrahedral material with a very short bond length ( $d$ ) and zero ionicity ( $\lambda = 0$ ). However, it should be possible to create materials with bulk moduli comparable to or even larger than that of diamond by reducing the bond length. One approach<sup>11</sup> is to attempt a synthesis of tetrahedrally coordinated insulators using carbon and nitrogen because the C-N bond length could be shorter than that of diamond. Even though a material of this kind will be partially ionic, the shorter bond length can over-compensate and increase  $B$ . Boron nitride is another superhard material of interest. The results for  $B$  using the above formula and a complete first-principles theory predicting  $B$  and the equation of state for boron nitride were tested<sup>12</sup> recently by direct experimental measurement. The predictions were found to be in excellent agreement with experiment.

Although the development of less-complex empirical approaches is likely to have a great influence on the development of useful materials, the emphasis at present is on the application and refinement of the first-principles theory. Research on the possibility of compressing hydrogen into a metallic state, the possibility of high-temperature superconductivity for metallic hydrogen, the existence of superhard systems and the general search for useful new materials can be aided by first-principles theory. Some materials have already been identified in this way and it is likely that more will be found as a result of theoretical predictions. □

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# Soap bubbles make serious physics

A new study of the evolution of rafts of soap bubbles confirms expectations and earlier measurements, but has the distinction of having been carried out with a photocopying machine.

Using a Xerox or some other brand of photocopying machine, with virtually no extra equipment, to make interesting physical observations is an achievement in itself. But Joel Stavans from the University of Pittsburgh and James A. Glazier of the University of Chicago seem also to have won some interesting physics from their primitive equipment (*Phys. Rev. Lett.* **62**, 1318; 1989). The issue is the evolution in time, by coalescence and other means, of a raft of soap bubbles which are small to begin with, but which get larger with the passage of time.

As with so many other topics, that of the evolution of a bubble raft goes back at least to J. von Neumann, among other things the author of a law describing the evolution of a single bubble embedded in a raft of them. It is important that, in circumstances such as these, bubbles are not circular or spherical but, rather, sit like prisms on polygonal bases. The neat way of fixing ideas is to imagine the experiment which Stavans and Glazier describe: put a raft of coloured soap bubbles in a very shallow plastic tray and seal a lid on top in such a way that the soap films stretch between the flat and horizontal upper and lower surfaces, giving each bubble (to a first approximation) the same height. Liquid collects at the lower boundaries between contiguous bubbles, so that the evolution of the bubbles in a raft may be followed simply by pressing the button on the photocopier at convenient intervals.

A bubble will grow or shrink by the diffusion of supporting gas from all nearest neighbours, which leads directly to von Neumann's law of bubble-growth that the rate of change of the volume of a bubble (or of its area as seen in a photocopied image) depends only on the number of vertical faces it presents to its neighbours in the raft, or on the number of sides of the polygon which forms the base. The starting-point for the argument is that a strictly regular array of bubbles each of which is hexagonal should be indefinitely stable, which leads directly to the rule  $da/dt = \kappa(n - 6)$ , where  $a$  is the projected area of the bubble,  $n$  the number of sides and  $\kappa$  a kind of diffusion constant.

The simplicity of this result is geometrical, deriving from the way in which both that rate of inward diffusion and the volume of the prism is determined by the total area of the vertical faces of the prismatic bubbles. A further assumption

is that the internal angles of each polygonal bubble-base are exactly  $120^\circ$ , which seems reasonable enough, given that each vertex of the polygon is a point at which three prismatic bubbles join: to a first approximation, they may be assumed to join symmetrically. But naturally this can be exactly true only when all bubble bases are regular hexagons. Otherwise, the sides of the polygonal bases will not be straight lines, but curves.

Stavans and Glazier are not primarily concerned with single bubbles, but with rafts of them, for which purpose they work with a distribution function called  $\varrho(n)$  which gives the frequency in any sample of the evolving bubble raft of bubbles with  $n$  sides, where  $n$  is necessarily an integer running from 3 to infinity.

Simple observation is said to show that the first stages of the evolution of the raft are rapid, with larger bubbles growing at the expense of smaller neighbours and the sidedness of particular bubbles changing upwards and downwards both by the disappearance of smaller neighbours and by a process in which vertical membranes shift so as to trade sides between one bubble and a neighbour. (Those planning to use their office photocopier for further investigations along these lines should beware of the edge effects, which complicate both the statistics and the underlying phenomenon.)

The authors explain how, when they made soap bubbles with helium gas, the general appearance of the raft thus formed was that of several patches of regular hexagonal bubbles interspersed with regions in which five and seven-sided polygons were mixed together. But the striking result, confirming earlier conjectures, is that there comes a point in the evolution of a bubble-raft in which the distribution function  $\varrho(n)$  becomes essentially invariant in the course of time. Moreover, the mean of the distribution (that is the average number of sides per bubble) is almost but not quite six, whatever the length of time for which the raft has been evolving (in one run, indeed, measurements extended over a day and a half). Only when the total number of bubbles becomes very small does the distribution go awry.

The same point emerges from measurements of the second moment of the distribution (essentially the weighted average of the squared deviation of the number of sides per cell from the average

value). Again, in at least two different systems, the value of the second moment fluctuated widely to begin with, but then quickly settled down to what seems to have been a constant value (estimated at  $1.4 \pm 0.4$ ). The interest seems to be that, as in fractal and chaotic systems, there seems to be an underlying scaling law which describes how, in an ageing raft of bubbles, the bubbles all get bigger, but the statistical properties of the quantity appropriately called sidedness remain essentially constant.

Departures from the rule that the internal angles of the basal polygons of each bubble should be  $120^\circ$  excite particular interest. What the measurements show is that bubbles with fewer than six sides have internal angles smaller or equal to  $120^\circ$ , but that bubbles with more sides have larger angles (although  $130^\circ$  seems to be the limit). Stavans and Glazier argue that the presence of angles differing from the expected value implies the accumulation of strain energy in the system, which allows them to generalize von Neumann's law (but only for those systems in which all the bubbles have the same number of sides) to take account of these departures from the expected. One practical conclusion seems to be that it is possible to understand why one bubble may grow at the expense of a neighbour with the same number of sides.

There remains one puzzle. Perhaps the simplest measure of the state of an ageing bubble raft is the average bubble area, which should, by simple integration of von Neumann's rule, be proportional to the time elapsed. But neither Stavans and Glazier nor their predecessors in the field have been able to measure anything like that — they quote a figure of  $0.59 \pm 0.11$ , as the exponent in a simple power-law dependence on the time.

Why should such a simple prediction apparently be so wide of the mark? It is natural that people should have gone haring after fanciful explanations to do with the scaling laws as bubble-rafts age. Prosaically, the authors suggest a more mundane explanation — that as a raft ages, more soap solution accumulates beneath the raft while there are thicker layers of it between membranes, the effect of which is to reduce the diffusion constant between neighbouring bubbles. It all goes to show that there are merits in even the least reliable photocopies.

John Maddox

# Dissecting a molecular motor

Peter Hollenbeck

THE organelles undergoing directed transport within eukaryotic cells are a strikingly diverse group. They vary widely in function and composition — consider membrane vesicles, pigment granules, mitochondria, and nuclei, for example — and range over at least two orders of magnitude both in their size and in their velocity of movement. Given this variation in the cargo, it is no surprise that cells use several different mechanochemical systems to generate force for movement. Two of these are well-characterized: myosin ATPase, which drives movement along actin filaments; and dynein ATPase, which generates unidirectional movement towards the 'plus' ends of microtubules. A third, kinesin, has not yet been so well characterized. On page 355 of this issue<sup>1</sup>, Scholey *et al.* describe the coordinated use of protein biochemistry, molecular genetics, antibody probes and electron microscopy to analyse the properties of kinesin, which should allow a comparison of these mechanochemical enzymes.

Although they interact with different filament systems, and have different kinetic properties, myosin and dynein have some interesting similarities<sup>2</sup>. In addition to a common structural motif (see figure), they share a crossbridging mode of force generation; they bind to the structure to be moved, thus crosslinking it to a filament; and hydrolyse ATP, transducing the energy thus liberated into movement along the filament. Several groups have dissected the kinesin molecule in attempts to fit its mode of action into this scheme, and Scholey *et al.*<sup>1</sup> are now able to assign the various activities of the kinesin molecule to different structural domains. They find that kinesin has an architecture very similar to myosin and dynein.

Kinesin, whose discovery and characterization I have discussed previously in News and Views<sup>3,4</sup>, is a ubiquitous protein motor which generates movement along microtubules in the opposite direction to dynein, and is believed to be involved in anterograde (or centrifugal) organelle transport. It is a heterotetramer, each molecule consisting of two ATP-binding heavy chains of relative molecular mass 110,000–140,000 (110–140K) and two 60–65K light chains<sup>5,7</sup>.

It appears in the electron microscope to have two globular heads at one end of a narrow stalk, similar to dynein and myosin, as well as a smaller pronged or fan-shaped region at the other end of the stalk<sup>8</sup>.

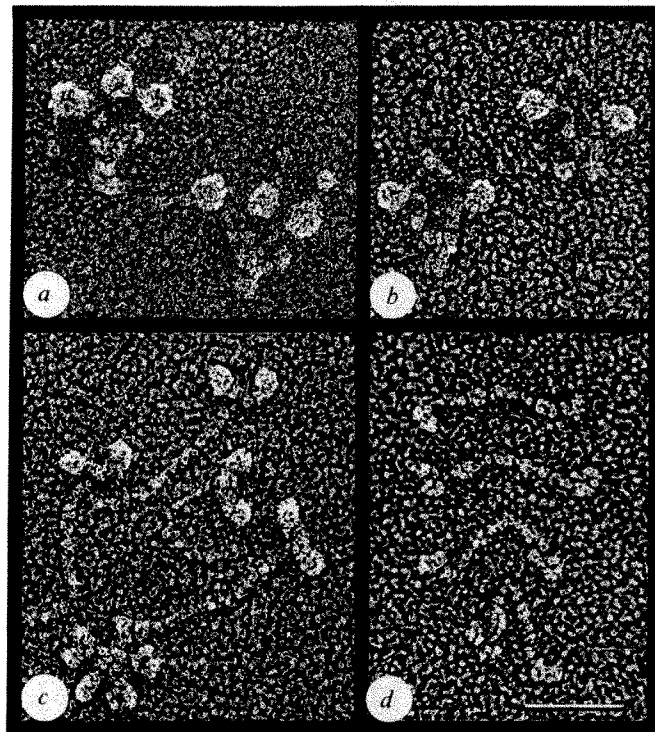
Degradation of the heavy chain with proteolytic enzymes yields a resistant 45K

The studies by Scholey and collaborators reported in this issue<sup>1</sup>, as well as recent work by Hirokawa *et al.* reported in the current issue of *Cell*<sup>11</sup>, combine several experimental approaches to map the location of specific functional domains on the kinesin molecule. Scholey *et al.* use freeze-etch electron microscopy of kinesin to show that an antibody which binds the 45K heavy-chain fragment and inhibits kinesin motility 'decorates' only the paired globular heads of the kinesin molecule. In addition, they use a series of

truncated kinesin heavy chains (produced by transcribing and translating truncated complementary DNA encoding the heavy chain) to demonstrate that the 45K fragment represents the amino-terminal one-third of the molecule, and that the inhibitory antibody binds near the carboxy-terminal end of the fragment, where the globular and  $\alpha$ -helical regions of the heavy chain meet. In a related study, Yang *et al.*<sup>12</sup> confirm that the amino-terminal portion of the heavy chain is necessary for microtubule binding.

Using quantitative morphometry of rotary-shadowed kinesin, Hirokawa *et al.*<sup>11</sup> show that monoclonal antibodies specific for the kinesin heavy chain decorate only the globular head regions, whereas those specific for the light chain decorate the fan-shaped region of the molecule at the opposite end of the stalk. These authors also report ultrastructural evidence that the globular heads interact with microtubules, whereas the feathered region, and perhaps a portion of the stalk, interacts with the moving structure.

A coherent picture of kinesin is emerging from these studies: the amino-terminal region of the heavy chains, residing in the globular heads, contains the microtubule-binding and ATP-hydrolysing capacities of the molecule; a flexible stalk connects this business end to an organelle-binding domain; and a domain important for the coupling of ATP



Electron microscope images of 'motor' molecules prepared by adsorption to mica, freeze-drying, and replication with platinum according to Heuser<sup>14</sup>. *a*, Two 3-headed ciliary dynein molecules prepared from *Tetrahymena* by U. Goodenough; *b*, two 2-headed cytoplasmic dynein molecules prepared from chick cells by E. Steuer; *c*, four myosin molecules from *Acanthamoeba*, one of which is attached to an anti-tail monoclonal IgM molecule (the asterisk-shaped entity in the lower left) (D. Kiehart); *d*, four kinesin molecules prepared from chick brain tissue by T. Schroer. Bar, 40 nm. (Courtesy of John Heuser.)

fragment which provides an important key to kinesin's fine structure. Kuznetsov *et al.*<sup>9</sup> analysed this fragment in detail and determined that kinesin's ATP hydrolysing and microtubule-binding activities reside there; in fact, the fragment shows elevated levels of both activities relative to the intact molecule. It is significant, however, that the fragment is not capable of generating movement in an *in vitro* assay. Ingold *et al.*<sup>10</sup> demonstrated that the fragment contains the binding sites for several monoclonal antibodies which inhibit kinesin-driven movement *in vitro* without inhibiting kinesin's microtubule-activated ATPase activity. This suggests that the fragment contains at least two functional domains — the ATPase site and an additional region distinct from the ATPase site but nonetheless essential for force generation.

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hydrolysis to force generation lies near the junction of the globular head and the stalk. The complementary DNA sequence of the heavy chain, determined by Yang *et al.*<sup>12</sup>, provides additional support for this picture; it predicts a large amino-terminal globular region containing an ATP-binding sequence, connected to a small carboxy-terminal globular region by a long stretch capable of forming an  $\alpha$ -helical coiled-coil<sup>12</sup>. This sequence also shows a proline-glycine break at approximately the region where a kink appears in electron micrographs of the stalk<sup>8,11</sup>.

So far, so good — but studies in which

organelle movements have been reconstituted *in vitro* from cell fractions suggest that a further functional domain of kinesin awaits identification. Schroer *et al.*<sup>13</sup> have shown pure kinesin alone is not enough to produce movement of salt-stripped membrane vesicles; additional factors are required. The sites of interaction between these potential regulatory factors and the kinesin molecule remain to be found. □

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## SUPERNOVA 1987A

# The remnant's rapid heartbeat

M. A. Alpar, I. Fushiki, F. K. Lamb, G. S. Miller, M.-G. Park and D. Pines

THE supernova explosion of February 1987 in the Large Magellanic Cloud has so far provided a wealth of data which, at least in broad outline, has confirmed our expectations of such an event and its aftermath. As the diffuse remnant has expanded and faded, astrophysicists have watched with mounting anticipation for any indication of the pulsar expected to have been formed in the explosion. The report by Kristian *et al.* in last week's issue<sup>1</sup> of optical pulses with a period of 0.5 ms is the first indication of such a pulsar. This report has aroused enormous interest among astrophysicists, as illustrated by the papers by Woosley and Chevalier<sup>2</sup> and Wang *et al.*<sup>3</sup> on pages 321 and 319 of this issue. It challenges almost all the main features of current theories of neutron-star formation, structure and evolution, and, most fundamentally, current models of nuclear interactions and the equation of state of matter at the highest densities. It seems to us that there are three possible interpretations of these results.

The most interesting possibility is that the pulses are produced by a neutron star rotating with a period of 0.5 ms, three times faster than any previously discovered. If so, this would strongly challenge the picture that has emerged from 20 years of research on neutron-star structure and the equation of state of dense matter. The near consensus has been that pulsars such as the Crab and Vela pulsars are spinning neutron stars of about 1.4 solar masses ( $M_{\odot}$ ) and with radii of 10–15 km. This reflects an equation of state describing neutron matter that is moderately stiff.

Models of pulsar glitches and post-glitch relaxation, the interpretation of the 35-day cycle of Her X-1 as neutron-star precession and deductions of the nuclear interactions from heavy-ion collisions<sup>4</sup>, all suggest that the equation of state of dense matter is relatively stiff. The only uniformly rotating models that predict neutron stars that are sufficiently compact

not to fall apart with a 0.5-ms rotation period are based on soft equations of state: the simultaneous demands that the equation of state be stiff enough for slowly rotating neutron stars with gravitational masses as high as  $1.4 M_{\odot}$  to exist and soft enough to permit the existence of a neutron star with a 0.5 ms rotational period are very constraining. The evidence for a stiff equation of state might be reconciled with a rotational interpretation of the reported pulsar if it had a very high central density, and if matter undergoes a phase transition at such high densities. This would then leave the question: why should SN1987A have left a particularly dense remnant?

The 0.5-ms rotation period would also constrain the dipole magnetic field strength  $B$  at the neutron-star surface. Electromagnetic dipole radiation has a power output proportional to  $B^2\omega^4$ , where  $\omega$  is the rotation rate. For this power to be less than the  $10^{36}$  ergs  $s^{-1}$  current total luminosity of the supernova remnant, the surface dipole field must be less than about  $10^9$  gauss (G). The magnetic fields of all of the relatively young (but not newborn) pulsars, of which the 1,000-year-old Crab pulsar is the paradigm, are estimated to be  $10^{11}$ – $10^{13}$  G. The pulsar implied by the report of Kristian *et al.*<sup>1</sup> would resemble these familiar objects only if the magnetic field can increase by a factor of about  $10^3$  within a thousand years — a possibility that was suggested some years ago, but which is highly speculative.

The alternative assignment of such a weakly magnetic pulsar to the class of other millisecond pulsars which have typical field strengths  $10^8$ – $10^9$  G challenges the widely accepted view that such pulsars are formed in low-mass binary systems (see the discussion by F. Graham-Smith<sup>5</sup> in News and Views last year). It is thought that such pulsars previously had periods of the order of seconds, and that their strong primordial fields have decayed. The combination of a weak magnetic field and

a high rate of accretion from the partner object in such binaries allows the neutron star to be spun up to a millisecond period. The periods, magnetic fields and ages of millisecond pulsars and rotation-powered pulsars in binary systems are consistent with this model. The location of some millisecond and binary pulsars in globular clusters, and the view that the fast quasi-periods observed in the low-mass X-ray binaries result from  $10^9$ -G magnetic fields in neutron stars rotating with millisecond periods, also fit this model.

It is conceivable that the weak magnetic fields in these seemingly old systems are primordial and do not result from the decay of stronger initial fields. Subsequent rapid accretion would then leave the star rotating with a millisecond period. But if the newly identified object in SN1987A is a newborn pulsar with both a weak magnetic field and a short spin period, this would undermine the model of spin up by accretion.

Woosley and Chevalier in this issue<sup>2</sup> propose that the pulsar was spun up to its millisecond period in the hours immediately following the supernova explosion. For this to occur, the neutron star's magnetic field must act to transfer angular momentum from the supernova envelope to the neutron star in a way similar to that supposed in the model for accretion from a binary partner. This explains, the authors suggest, why the new pulsar has the same field-period correlation as the other millisecond pulsars. But the high accretion rate necessary in this model means that the mechanism for generating the observed pulses must be different from that in canonical pulsars. Also, one might expect the accreting material to smear the pulses by scattering.

If the 0.5-ms period is established to be a rotational period and this pulsar turns out to be in the same class as the previously known millisecond pulsars, a fundamental revision of our ideas of pulsar classes, statistics and evolution will be required. Given a neutron star with such an unusual structure and spin period, the natural question for astronomers will be whether the progenitor star and the supernova event itself were peculiar. Do such blue giants have unusually weak magnetic fields, rapid rotation rates or peculiar internal structures? Even if systematic differences are identified, supernova theorists will face the challenge of linking them to distinct features of the stellar core that collapses to become the neutron star.

An alternative possibility proposed by Wang *et al.* in this issue<sup>3</sup> is that the neutron star is ringing, or vibrating, with a period of 0.5 ms. A particular attraction of this idea is that it does not require a new and different kind of object or a radical revision of our current understanding of neutron stars and pulsars. Indeed, a 0.5-ms radial vibration would be typical of neutron stars

with reasonable masses and equations of state. Historically, neutron-star vibrations were rejected as an explanation of pulsars because vibration periods are much shorter than the vast majority of observed pulsar periods, and because the observed pulses must persist for thousands to millions of years. Such persistence is natural for neutron-star rotation, but difficult for vibrational oscillations, which are expected to be damped within a few hundred years. However, this need not pose a problem for a newborn pulsar, because radial modes excited at the time of formation may not yet have been damped.

Wang *et al.* propose that some of the vibrational energy is converted into the observed light pulses by the formation of a shock front as the vibrations propagate into a thin surface region of the star where the density and sound speed decrease very sharply. The model requires the shock to accelerate iron ions to energies of 100 GeV; the ions subsequently emit cyclotron radiation without losing much of their energy to electrons or lighter nuclei. A surface magnetic field of  $10^{12}$  G is needed for the iron ions to emit radiation in the optical band. Light nuclei and electrons would be relativistic at 100 GeV, and hence would require lower magnetic fields to emit predominantly in the optical; their synchrotron lifetimes would then be longer than the observed pulse period, washing out the pulsations. Wang *et al.* postulate that the field is  $10^{12}$  G, in which case the rotation period must be longer than 0.1 s or the dipole radiation power would be more than the current supernova luminosity. Stable polarized pulses indicating rotation with a period in this range would provide strong support for the radial-oscillation hypothesis.

Confirmation of the vibrational mechanism, which would not challenge the existing theory, would focus new attention on aspects of neutron stars such as the structure of their surface layers, the propagation of radial oscillations and shocks, and the coupling of such motions to rotation, non-radial modes and possibly to gravitational radiation. There would also be renewed interest in the generation of optical pulses, either through the mechanism proposed by Wang *et al.* or by other means. Nevertheless, it is hard to see why the harmonic content of the observed pulses should closely match that of the Crab pulsar, if the pulsation mechanism is indeed so different. In particular, if the pulse has two peaks, a main pulse and an interpulse, as is suggested by the relative strengths of the fundamental and first harmonics, then this may be difficult to model with vibrationally driven pulsations.

Whatever the explanation for the pulses, there seems also to be an 8-hour sinusoidal modulation of the pulse period,

which poses additional problems. Precession of the neutron star cannot account for the large phase offset observed. Proposals that the modulation is the result of timing noise must confront the comparatively noiseless sine curve of the observed data: it is possible, but unlikely, that this has been produced by a random process. The idea that the modulation arises from a Doppler shift as the pulsar orbits a small, unseen companion, raises the problem of explaining how a companion comes to be so close to the neutron star. It is very unlikely that material left over from the progenitor star would be able to cool and condense into a gravitationally bound companion. Had the companion existed independently before the explosion, it should have had to exist within the progenitor star. But perhaps a companion was produced as the result of spin-up of the core during the supernova implosion.

The third possibility, and the least appealing one, is that the observations are

not what they seem, and that no pulsar has actually been detected, despite the strong signal observed and careful analysis. This would save us the trouble of revising our cherished beliefs, but would leave us wondering uncomfortably what was observed, if not a pulsar? Still, until the report of Kristian *et al.* is confirmed by further observations, this is a possibility that must be considered. Should the report not be confirmed, the questions which it has raised may well remain unresolved. This would surely be a disappointment, in view of the exciting challenges the initial report has posed. □

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## METEORITES

# Unique find from Antarctica

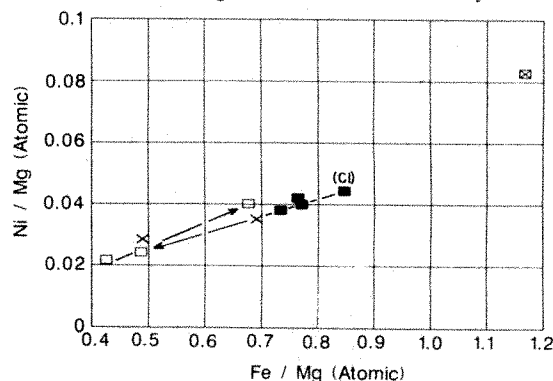
Robert T. Dodd

METEORITES, it is generally agreed, are mostly pieces of pre-planetary material that both come from and were formed in asteroidal parent bodies. This interpretation makes meteorites uniquely important as witnesses to events in the primitive solar nebula, but it also raises a serious question about how typical they are of nebular material. Well-classified meteorites come from no more than 20 of the thousands of asteroids that have been described. Worse, spectral reflectance comparisons suggest that the most abundant meteorites — ordinary, or common, chondrites — come from very rare asteroids: the dark objects that are most numerous in the asteroid belt have, in contrast, yielded few meteorites. So it seems that our sample is incomplete and strongly biased towards those asteroids that are now well-placed to deliver meteorites. If so, meteorites recovered from the Antarctic ice cap, some of which fell a million or more years ago, may both introduce us to new kinds of meteorites and provide fresh insights on old problems. Allan Hills 85085, a thumb-sized Antarctic meteorite described recently in three consecutive papers in *Earth and Planetary Science Letters*<sup>1–3</sup>, does both.

Despite suggestions to the contrary<sup>4</sup>, ALH85085 seems to be a chondrite: it contains both the spherical-to-rounded objects — chondrules — from

which such meteorites take their name and inclusions of highly refractory calcium–aluminium silicates (CAI) like those found in many carbonaceous chondrites. It is, however, sufficiently different from other chondrites to make its classification difficult. Its chondrules are few, small, and accompanied by much fragmental material. According to Scott<sup>1</sup> and Weisberg *et al.*<sup>2</sup>, its silicate particles are very closely sized, and the associated oblong metal particles appear to have a preferred orientation<sup>2</sup>.

ALH85085 lacks the extremely fine-grained, dark matrix material that surrounds chondrules in other primitive chondrites, but it contains lumps of similar material which at least resemble<sup>1,2</sup> and, according to Grossman *et al.*<sup>3</sup>, may in fact



Metal–silicate fractionation trends in chondrites. Mean ratios have been normalized to the Si/Mg ratio in CI chondrites to remove the effect of refractory element fractionation. Data from refs 3 (ALH85085) and 7 (chondrite groups). ■, C groups; ×, E groups; □, O groups; ☒, ALH85085.

## CORTICAL PHYSIOLOGY

## Is grandmother an oscillation?

Michael P. Stryker

be fragments of chondrule-poor carbonaceous chondrites. The meteorite also differs from other chondrites in chemical composition<sup>1,2</sup>: it is enriched in iron, nickel and other siderophile elements and is deficient in sulphur, alkalis and other elements that vaporize at low temperatures (volatiles).

Those who have studied ALH85085 agree that it is a unique meteorite, whose composition and mineralogy suggest that it is most closely related to two unusual carbonaceous chondrites, Renazzo and Al Rais. Whether it should be grouped with those meteorites<sup>3</sup> or left unclassified<sup>1,2</sup> is less certain. An oxygen isotope analysis or a cosmic-ray exposure age, neither of which is now available, may answer that question.

Of the many intriguing features of ALH85085, its enrichment in siderophile elements and depletion in volatile elements are most significant, for they seem to answer a long-standing question. Almost two decades ago, Larimer and Anders proposed<sup>4</sup> that other kinds of chondrites evolved from material similar to the chondrule-free CI carbonaceous chondrites (*sic*) by extraction of metallic nickel-iron and refractory calc-aluminous silicates.

Their interpretation, perhaps modified to allow for two episodes of metal fractionation<sup>5</sup> (see figure), is plausible and widely accepted, but it leaves a great deal of siderophile material unaccounted for. Hence the discovery of a chondrite that contains excess metal of about the right composition<sup>2</sup> is important, both for the chemical evolution of chondrites and for the origin of other objects — the Earth and Moon included — that also appear to have formed from atypical chondritic material<sup>1</sup>.

To date, most meteorites from Antarctica have turned out to be samples of known types or modest variations on familiar themes. If the Earth receives a biased sample of the meteorite sources, it seems that it has done so for at least a million years. Yet the ice cap has already yielded many unusual and important objects, which include the first lunar meteorites and additions to a small collection of meteorites from Mars. ALH85085 shows that even its smallest gifts may open new windows on the origin of the Solar System. □

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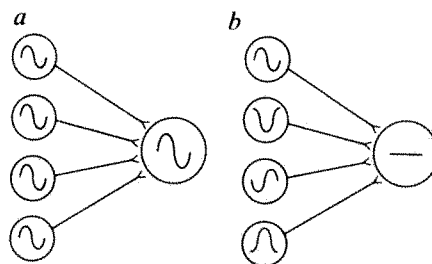
THE realization that visual information is processed through successive stages in many separate areas of the cortex has led to a dilemma. How are the distributed representations of the visual features that have to do with a single object in the world put together so that they can create a perception or influence action? That is, when a green furry tennis ball and an ebony billiard ball are seen in the same part of the visual field, how can the colour, motion and texture properties of each ball be associated, so that the black colour and smooth texture of the billiard ball is not ascribed to the tennis ball? Three new papers<sup>1-3</sup> from two laboratories, one on page 334 of this issue<sup>2</sup>, provide a clue.

Several proposals have been put forward to solve this problem. A now-classic notion is that significant combinations of features are hierarchically extracted and combined in specific cortical areas specialized for the recognition of certain classes of objects<sup>4,5</sup> or, at the extreme, in the receptive fields of single neurons<sup>6</sup>. In some higher cortical areas, there could be, for example, 'grandmother' neurons responding selectively to the precise combinations of visual features that are associated with one's grandmother. Either such cells would not be able to signal the location of stimuli very accurately or there would have to be a separate grandmother cell for each region of the visual field. It is also not clear how the many different features that might be associated with one's grandmother could be combined in any very selective way without a 'combinatorial explosion' in the numbers of cells required<sup>7</sup>. This would lead to the sort of problem Little Red Riding Hood had when her grandmother cells failed to discriminate the wolf's grey fur, sharp white teeth and heavy breathing from her grandmother's normal benign appearance.

These shortcomings have led to an alternative notion — that the representations in the brain of various visual properties of objects in the world are combined only transiently, rather than in fixed receptive fields, in some way that makes the conjoint output of different property-specific detectors available to the mechanisms for perception or action. Some years ago, Crick<sup>8</sup> suggested a mechanism by which the neurons of thalamic reticular nucleus below the cortex could unify the perceptual qualities represented in different cortical areas. He proposed that a neural 'searchlight' would simultaneously illuminate all the neurons that are activated by the same object in the world.

The recent work from Gray, Singer and colleagues, reported in this issue<sup>2</sup> and else-

where<sup>1</sup>, and from Eckhorn and colleagues<sup>3</sup>, raises a related possibility: that neurons in the visual cortex activated by the same object in the world tend to discharge rhythmically and in unison. Such a one-note neural harmony could, in principle at least, provide the neurons at higher cortical levels with stronger inputs so that they associate the activities of lower-order neurons with one another (see figure). If the discharges of the texture-, colour-, depth- and movement-sensitive neurons



Four lower-order neurons providing input to one higher-order neuron. *a*, Neuronal activity in the lower-order neurons is shown oscillating in phase. The resulting postsynaptic potentials sum in the target cell, producing a large oscillation in its membrane potential. At the peak of this oscillation, the membrane potential of the higher-order neuron would exceed the discharge threshold, and the cell would fire rhythmic, high-frequency bursts of spikes. *b*, The activities in the lower-order neurons do not oscillate in phase, so the higher-order neuron receives a more nearly constant input. The resulting steady membrane potential in the higher-order neuron either would be below the threshold for spike discharge or would, in any case, not allow it to discharge at the high frequencies characteristic of neuronal responses to sensory stimuli.

concerned with the tennis ball were to oscillate in phase with one another and out of phase with the billiard ball responses, this might enable perceptual mechanisms to assign the furry texture, green colour and blinding speed to the one object and the smooth texture, ebony colour and moderate speed to the other. I have made a simple estimate that, with reasonable assumptions about the duration of postsynaptic potentials, such a mechanism would enable higher-order cells to distinguish inputs from one set of neurons from those of 10 or more sets of neurons, if each set responds to different objects in the world at different phases or frequencies. If, instead of summing, synaptic inputs can interact multiplicatively<sup>9</sup>, cortical cells could detect phase-locked activity with even more sensitivity.

This new evidence provides only the first hints that the visual cortex uses such mechanisms. Gray and Singer<sup>1</sup> find that visual stimulation can cause many neurons in visual cortex to discharge their action

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potentials rhythmically at about 40–50 Hz in very lightly anaesthetized (and, as reported in an abstract elsewhere, awake) animals. This rhythmicity is accompanied by an oscillation in the extracellular field potential and seems to originate in the cortex, as it was not evident in recordings from the main input to the visual cortex from the thalamus. Eckhorn and colleagues<sup>1</sup> find that oscillatory field potentials evoked by some visual stimuli are in phase even between the two primary visual cortical areas (17 and 18) in the cat and that the field potentials in one area are in phase with the action potential discharges in the other. In their paper in this issue<sup>2</sup>, Gray *et al.* report that oscillations in the discharge are commonly in phase for neurons with overlapping receptive fields, irrespective of their selectivity for a particular stimulus orientation<sup>3</sup>. But when the authors record at sites more than 2 mm apart, where receptive fields no longer overlap, they find that oscillations are rarely in phase except for neurons with the same orientation specificity.

The most surprising observation comes from two cases in which pairs of recording sites were located half a visual cortex (7 mm) apart. Receptive fields of the neurons at the two sites had a common orientation specificity and were aligned so that they could be stimulated by a single long bar of light (see Fig. 3b of ref. 2 on page 336). Neuronal discharges at the two sites were well correlated only when the cells were stimulated with a single long bar. When they were simultaneously activated by two shorter bars that did not bridge the gap between the two receptive fields, the correlation disappeared. In that sense, the correlated discharge can be considered as depending on the global property of the stimulus — whether it is a single or two different objects.

Numerous questions are raised by these tantalizing observations. Where does the rhythm come from? Many workers have dismissed findings of rhythmic discharge in the central nervous system as artefactual, as rhythmicity can be induced by certain anaesthetics or by damage. Further work demonstrating that such phenomena are definitely present in awake animals will be needed to satisfy these critics. Even if not an artefact, the oscillations may be epiphenomena:

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observations of rhythmicity and its correlation with particular stimuli do not allow the conclusion that the nervous system makes use of such information. This point will be difficult to address except by demonstrating that many global aspects of visual stimuli are accompanied by correlated rhythmic discharges. In particular, it would be intriguing to know whether a single bar or edge produces such correlated activity when and only when it appears as a single object; a physical discontinuity that does not degrade the perception of the bar as a single object, as when the bar is partially occluded<sup>4</sup>, should not destroy the correlated oscillations. It will be even more intriguing to see whether neurons in the different cortical areas specialized for colour, depth, motion and so on exhibit correlated, rhythmic discharges when

## PRIONS

# Sheep disease in human clothing

Charles Weissmann

THE identification of polymorphic markers linked to a genetic disease is an important goal both for geneticists and for molecular biologists. The former make use of the association to determine individuals at risk for the disease, the latter to hunt for the gene causing it. Because it is based on trial and error, the search is often protracted. But this is not the case in the familial (ataxic) form of Gerstmann-Sträussler syndrome, a transmissible neurodegenerative disease so rare that it is not mentioned in the human geneticist's bible, *Mendelian Inheritance in Man*<sup>1</sup>. Guided by their work on scrapie, a related brain disease of sheep, Prusiner and colleagues, as they report on page 342 of this issue<sup>2</sup>, have in one fell swoop identified a polymorphic marker tightly linked to this syndrome. At the same time, these authors provide persuasive evidence that a surface membrane protein in neurons called PrP has a central role in the pathogenesis of the human disease.

Gerstmann-Sträussler syndrome and Creutzfeldt-Jacob syndrome in humans, as well as scrapie and bovine spongiform encephalopathy in animals, belong to a class of slow, transmissible, lethal brain diseases caused by an unusual kind of pathogen which Prusiner<sup>3</sup> called a prion. The main pathological features of prion-associated diseases are vacuolation of neurons, proliferation of glial cells and occurrence of amyloid plaques containing a form of PrP. The diseases have extended incubation times that can be greater than 30 years.

Scrapie has been used as a model for prion diseases because it has been transmitted to hamster and mouse, making it suitable for experimentation. Because scrapie infectivity is very resistant to heat

they respond to the same stimulus in the real world.

Observations of this type could provide compelling evidence that perceptual mechanisms of the brain do engage in the analysis of brain rhythms. The suggestion that coherent oscillations in activity identify the members of subsets of a large neuronal population may, however, still be an important one, even if these subsets are not closely tied to perception. Exploring the rhythms of the brain, revered by the pioneers of electroencephalography but now mostly dismissed as irrelevant to neural information processing, may even come back into fashion. □

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and nucleolytic agents but sensitive to procedures that modify or destroy proteins, it is unlikely to be caused by a conventional virus, plasmid or viroid. The unusual resistance of the infective agent to ultraviolet and ionizing radiation led not only to the conclusion that the mass of the agent is as low as 55,000 (55K) but also that it is devoid of nucleic acid altogether<sup>4</sup>.

Scrapie infectivity was purified several thousandfold from infected brain extracts by a procedure involving proteolysis under conditions where much of the protein but not infectivity is destroyed<sup>5</sup>. The resulting preparations contain a protein of relative molecular mass 27–30K, designated PrP 27–30, as the major component; although they are not free of nucleic acids<sup>6</sup>, no scrapie-specific polynucleotide has yet been identified in them, particularly no PrP-encoding sequence<sup>6</sup>. Surprisingly, PrP turned out to be a host-specified protein, encoded by a single exon of a unique host gene<sup>7</sup> and to be expressed both in normal and diseased animals<sup>8</sup>. Brains of scrapie-infected hamsters contain two forms of PrP: the scrapie (PrP<sup>Sc</sup>) and cellular (PrP<sup>C</sup>) isoforms. Both proteins have a mass of 33–35 K, but they have different physical properties. PrP<sup>C</sup> is anchored to the cell surface by a glycosyl phosphatidylinositol linkage<sup>9</sup> and can be solubilized with ionic detergents or phospholipase C, whereas PrP<sup>Sc</sup> cannot. PrP<sup>C</sup> disappears under proteolytic conditions while PrP<sup>Sc</sup> loses only an amino-terminal peptide to yield a protein of mass 27–30K called PrP 27–30. This is why PrP 27–30 is recovered from scrapie-infected but not from normal brains.

So far, no differences in the primary structure of PrP<sup>C</sup> and PrP<sup>Sc</sup> have been detected<sup>6, 8–10</sup>, nor have any differences

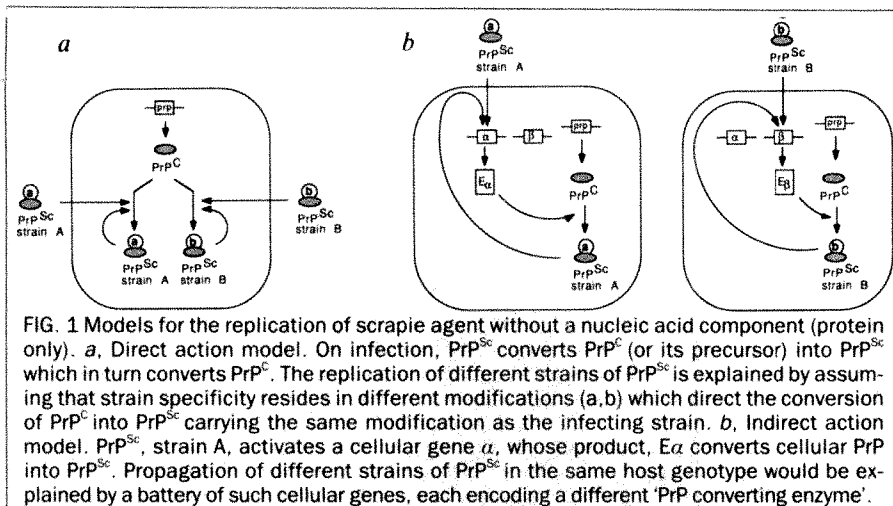


FIG. 1 Models for the replication of scrapie agent without a nucleic acid component (protein only). *a*, Direct action model. On infection,  $\text{PrP}^{\text{Sc}}$  converts  $\text{PrP}^{\text{C}}$  (or its precursor) into  $\text{PrP}^{\text{Sc}}$  which in turn converts  $\text{PrP}^{\text{C}}$ . The replication of different strains of  $\text{PrP}^{\text{Sc}}$  is explained by assuming that strain specificity resides in different modifications (a,b) which direct the conversion of  $\text{PrP}^{\text{C}}$  into  $\text{PrP}^{\text{Sc}}$  carrying the same modification as the infecting strain. *b*, Indirect action model.  $\text{PrP}^{\text{Sc}}$  strain A, activates a cellular gene  $\alpha$ , whose product,  $\text{E}\alpha$  converts cellular  $\text{PrP}^{\text{C}}$  into  $\text{PrP}^{\text{Sc}}$ . Propagation of different strains of  $\text{PrP}^{\text{Sc}}$  in the same host genotype would be explained by a battery of such cellular genes, each encoding a different 'PrP converting enzyme'.

been found between PrP genes or messenger RNAs from normal and infected brains with respect to structure or copy number. The physical differences between the two proteins are therefore attributed to a post-translational modification. It is known that PrP expressed in uninfected cells from the cloned PrP gene does not cause scrapie<sup>11</sup>.

Strains of scrapie prions differing with respect to incubation time and brain vacuolation pattern<sup>12,13</sup> can be passaged many times in the same inbred mouse strain and retain their characteristic properties. Interconversion of certain scrapie strains occurs reproducibly, particularly on transfer from one host to another<sup>15</sup>. Thus, scrapie prions have heritable properties. Incubation time is, however, also determined by a host gene designated *sinc* or *prn-i*<sup>12,14</sup>; remarkably, the *prn-i* gene is closely linked or perhaps identical with the PrP gene<sup>14,15</sup>. Mice with short and long incubation times have different amino acids in positions 108 and 189 of the PrP protein, suggesting that the structure of host PrP affects incubation time.

There are two main hypotheses for the

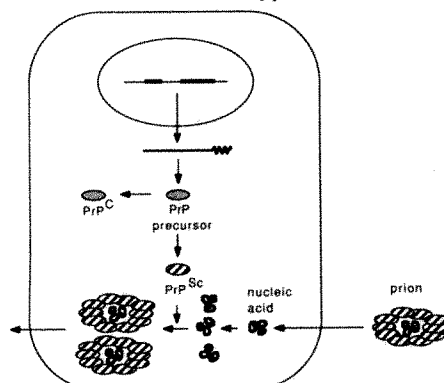


FIG. 2 Model for the replication of scrapie agent containing  $\text{PrP}^{\text{Sc}}$  and a nucleic acid. A nucleic acid associated with host-encoded  $\text{PrP}^{\text{C}}$  infects the cell. After replication of the nucleic acid, infectious particles are assembled from nucleic acid and PrP. Conversion of  $\text{PrP}^{\text{C}}$  to  $\text{PrP}^{\text{Sc}}$  is caused by the association with the nucleic acid, or (as here)  $\text{PrP}^{\text{C}}$  or its precursor may first be converted to  $\text{PrP}^{\text{Sc}}$ .

nature of the prion. First, the 'protein only' hypothesis proposes that the prion is a protein or protein derivative, devoid of nucleic acid<sup>3,4</sup>, for which  $\text{PrP}^{\text{Sc}}$  is the most likely candidate. The primary PrP translation product would have to be converted into the modified, infectious form in the  $\text{PrP}^{\text{Sc}}$ -infected cell. This conversion might be directly catalysed by  $\text{PrP}^{\text{Sc}}$  (ref. 16). Each scrapie strain would be represented by a different variant of  $\text{PrP}^{\text{Sc}}$  which modifies one precursor into a product resembling itself (Fig. 1a). Strain conversion on passaging in a different host could be accounted for by polymorphic variants of the resident PrP gene. In a more complex model, conversion of  $\text{PrP}^{\text{C}}$  to  $\text{PrP}^{\text{Sc}}$  would be catalysed by a host-encoded 'converting enzyme' induced by  $\text{PrP}^{\text{Sc}}$ . The variety of scrapie species would be explained by a battery of distinct converting enzymes, each activated by the cognate strain of  $\text{PrP}^{\text{Sc}}$  (Fig. 1b).

The second, 'nucleoprotein' hypothesis, postulates that the prion consists of a small nucleic acid and host-encoded protein<sup>3,17</sup> for which  $\text{PrP}^{\text{Sc}}$  is the most likely candidate (Fig. 2). Strain differences are ascribed to variations in the nucleic acid, just as in the case of viroid RNA.

How do the new data on Gerstmann-Sträussler syndrome reported in this issue<sup>2</sup> affect our knowledge of prion diseases? In two apparently unrelated families, one in the United States and one in the United Kingdom, Prusiner and colleagues find that an ataxic form of the syndrome is linked to a change in codon 102 in one of the PrP alleles from proline to leucine. Prusiner and colleagues do not find this mutation in 100 normal individuals or in 15 individuals suffering from other forms of inherited or sporadic prion diseases. Using the data from both pedigrees, assuming dominant inheritance and making certain assumptions regarding age-dependent penetrance as well as disease and marker frequencies, the authors calculated a lod score of about 3.3, which is highly significant evidence for linkage. As Gerstmann-Sträussler

syndrome is a very rare transmissible disease, familial occurrence could have been interpreted as being due to pre- or perinatal infection with an unknown agent. The new linkage data show that a genetic component is essential for acquisition or manifestation of the disease.

A more fascinating consideration is that if the two families that suffer the syndrome are, as suspected, unrelated, and the identical PrP mutations arose independently, the amino-acid substitution would not only represent a genetic marker but also play an essential role in pathogenesis. This interpretation is strongly supported by an impending report describing two unrelated Japanese families with the ataxic syndrome that show the same proline-to-leucine substitution<sup>18</sup>. It could be argued in the 'protein only' hypothesis that the amino-acid replacement greatly increases the probability of spontaneous conversion of  $\text{PrP}^{\text{C}}$  to (an as yet putative)  $\text{PrP}^{\text{GSS}}$  which would then behave like a transmissible agent (it will be interesting to learn whether in these heterozygous patients  $\text{PrP}^{\text{GSS}}$  contains only the protein with the amino-acid substitution). One might further argue that in sporadic cases of Gerstmann-Sträussler syndrome and Creutzfeldt-Jacob disease a somatic mutation gives rise to a variant  $\text{PrP}$  with enhanced capacity to convert into  $\text{PrP}^{\text{GSS}}$  or  $\text{PrP}^{\text{CJD}}$ .

Although the new results<sup>2</sup> tip the scales in favour of the protein only hypothesis, they do not exclude the possibility that a nucleic acid is part of the prion. Taking into account the occurrence of sporadic cases of the diseases, such a nucleic acid or prion would have to be widespread in the population, but with low penetrance in the absence of predisposing PrP mutations. Ultimately, the question as to the nature of the prion should be resolved by generating infectivity *in vitro*, either from pure, non-infectious  $\text{PrP}^{\text{C}}$ , from a nucleic acid, or from a combination of the two. □

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# Observations beyond the limit

Demosthenes Kazanas

CYGNUS X-3 is one of the brightest and most extraordinary objects in our Galaxy. It was first discovered as an X-ray source in 1966 and identified as the source of a giant radio outburst in 1972. Subsequent observations of Cyg X-3 have heralded the extension of the energy range for astronomy first to teraelectron volts (1 TeV =  $10^{12}$  eV) and then to petaelectron volts (1 PeV =  $10^{15}$  eV). Now Cassidy *et al.*<sup>1</sup>, using the 'Fly's Eye' detector in Utah, have observed Cyg X-3 at higher energies still — exaelectron volts (1 EeV =  $10^{18}$  eV) and above. The authors estimate the probability that the excess of cosmic rays at this energy observed from the direction of Cyg X-3 could occur by chance in a uniform distribution of showers is only  $6 \times 10^{-4}$ .

Collisions of high-energy primary cosmic rays in the upper atmosphere initiate showers of secondary particles which can be observed by astronomers. The Fly's Eye detector used by Cassidy *et al.*<sup>1</sup> maps the nitrogen fluorescence excited in the atmosphere by such showers and has a coverage of  $2\pi$  steradians. Since November 1986, the operation of two such detectors has permitted three-dimensional reconstructions of the showers. The TeV cosmic rays are observed via the Cerenkov (electromagnetic shock wave) radiation emitted by charged secondaries. And PeV rays initiate showers of electrons (and muons) observed directly by detectors on the ground. Each technique has a resolution of  $1^\circ$ , so that point sources can be identified.

## Interest and scepticism

Since its introduction in the early 1980s the field of astronomy at energies above 1 TeV has been viewed with both interest and scepticism<sup>2</sup>. The interest stems from the apparently counterintuitive fact that a large fraction (a tenth) of the luminosity of galactic accreting objects is emitted at energies above 1 TeV — well above the potential energy at the surface of even a neutron star — and in some cases up to 1,000–10,000 TeV. The scepticism arises from the rather low statistical significance of the observations owing to the small number of particles arriving from these sources at these energies: at such high energies, only a few particles are needed to contribute significantly to the total luminosity of an object. It was hoped that new larger detectors would settle the difficulties with statistical significance.

Unfortunately, observations of Cyg X-3 with one of these detectors reported recently<sup>1</sup> provide only upper limits to the flux at PeV energies, and the observers arrived at the unsatisfactory conclusion

that PeV emission ought to be episodic. This conclusion is nonetheless strengthened by observations of another source, Her X-1, an X-ray pulsar (period 1.2378 s), which has also been detected in the TeV and PeV range<sup>3,6</sup>. These observations indicate that at energies of 1 TeV or more, the signal from this source appears in bursts lasting anything from a few minutes to a hundred. A search for periodicities in these bursts indicates a period of 1.2357 s — an intriguing result, as this is significantly different from the period of the X-rays. To compound the puzzles, the muon content in the PeV showers is inconsistent with the assumption that they are initiated by photons (if they were, there should have been ten times fewer muons).

Instead, the muon content is consistent with that expected from hadron-initiated showers. But any such particles, if electrically charged, would be disturbed while traversing the turbulent interstellar magnetic field, so that the observed directional and temporal coherence of the showers could not have been preserved. And there are no known stable neutral particles that could fit the description.

Amid all these puzzles and problems there has been a significant recent advance which may put the whole field on a much firmer ground<sup>7</sup>: the detection of a statistically highly significant (nine-standard-deviation), constant signal of 1-TeV photons from the Crab nebula (background showers initiated by cosmic-ray hadrons were identified and subtracted). This is by far the most significant very-high-energy detection and was achieved by imaging the showers' Cerenkov radiation, a technique which allows the rejection of 98 per cent of the background events. The importance of this result lies in that it provides a weak ( $10^{34}$  erg s<sup>-1</sup>) but steady 'standard candle' against which future experiments will be calibrated.

What can be learned from all these observations? First, a large fraction of the accretion energy in many X-ray sources is converted (albeit sporadically) into relativistic protons with energies greater than 1 TeV (it is generally agreed that it is too difficult to accelerate electrons to these energies) which subsequently produce the observed high-energy radiation through nuclear collisions. Several models for these sources have been proposed (see ref. 8 for a review) which, however, are rather unconstrained owing to the scarcity of photons and lack of repeatability of the observations. Their most important feature is the estimate of the maximum energy expected (about  $10^{16}$ – $10^{17}$  eV) by the acceleration mechanism favoured by each model (shock acceleration near the

compact object or large-scale static electric fields).

Given their simplicity, the agreement of these models with the observations in the  $10^{15}$ – $10^{16}$ -eV range is rather remarkable. With the maximum particle energy already set by the models, it would seem that the new observations of Cassidy *et al.*<sup>1</sup> are in direct conflict with theory, especially if the observed particles are photons, which would have to be generated at the source by particles of energy  $10^{19}$  eV. Cassidy *et al.* point out, however, that the observed showers could be due to neutrons. (The Fly's Eye cannot distinguish between photon- and neutron-induced showers; also neutrons, although unstable, can get to Earth at these energies without much attenuation from decay.) Indeed, Ellison and I have proposed<sup>9</sup> that neutrons are copiously emitted from these objects and could be observed on Earth.

## High-energy tail

Furthermore, if the spectrum of the protons accelerated in the source decreases exponentially above the energy of maximum intensity suggested by the current models ( $10^{17}$  eV), the energy flux at 1 EeV should be  $\exp(10) \approx 10^4$  times smaller than that at lower energies, in rough agreement with the observations obtained with the Fly's Eye. These observations might therefore probe the high-energy end of the particle distribution that produces the radiation at PeV energies.

As for the muon content of the showers, the situation remains confused. An obvious solution is an unknown strongly interacting particle; more appealing, to my taste, is the recent proposal<sup>10</sup> that the quantum-chromodynamic structure of the photon causes an increase in the photo-production cross-section for particle collisions at energies of 0.5 TeV in the centre of mass. At these energies, the photons can interact with nuclei in the atmosphere by the nuclear strong force, a process which may compete with the electromagnetic ones considered so far in the shower development and enhance the muon content. These showers might hence be pointing to new physics at these energies, to be investigated with further observations and detailed modelling. □

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## GRAVITATIONAL PHYSICS

## Fifth force remains elusive

Christopher Stubbs

COULD there exist a fundamental interaction that has until recently escaped detection? This notion was addressed at a recent workshop\* where participants assessed the current status of the 'fifth force'. The geophysical evidence for a violation of the inverse-square law of gravitation, one way such a force could be manifested, emerged weakened from the meeting, and the results reported from experiments investigating the universality of free fall were in accord with Newton's hypothesis. Nevertheless there remain some tantalizing indications that a new force exists, and given the advances made in the past year the consensus was that experimental effort should continue.

The suggestion 3 years ago<sup>1</sup> of a previously unobserved fifth force prompted a flurry of experiments designed to test the idea. Two types have so far been performed: tests of the weak-equivalence principle comparing the forces exerted on two different substances by some attractor; and geophysical tests of the inverse-square law that seek deviations from the newtonian prediction in the vertical gravity profile. Results are typically expressed in terms of a 'Yukawa' modification to newtonian gravity

$$V_s = a \left( \frac{q_s}{\mu} \right) \left( \frac{q_s}{\mu} \right) G \frac{M_1 M_2}{r} e^{-r/\lambda}$$

where  $\lambda$  and  $\alpha$  are the range and dimensionless strength of the interaction and  $GM_1 M_2/r$  is the familiar newtonian expression relating the gravitational potential between two masses  $M_1$  and  $M_2$  to their separation  $r$  and the gravitational constant  $G$ .

The relevant 'charge' per atomic mass unit  $q_s/\mu$  is generally assumed to be some linear combination of  $B/\mu$  and  $L/\mu$ , with  $B$  and  $L$  baryon (nucleon) and lepton (electron) number for the material, respectively. Experiments testing the equivalence principle compare the accelerations of materials presumed to differ in  $q_s/\mu$ ; and the geophysical measurements seek the effects of the exponential term in the potential. The original fifth-force hypothesis favoured  $q_s=B$ ,  $\alpha \approx 0.01$  and  $10 \text{ m} < \lambda < 1,000 \text{ m}$  (see the discussion by De Rújula in News and Views<sup>2</sup>).

Statistically, the most significant geophysical evidence for a departure from newtonian gravity comes from measurements of  $g(z)$ , the vertical profile, up a 600 m television transmission tower<sup>3</sup>. It is claimed that these measurements differ from the newtonian prediction calculated from an array of surface gravity measure-

ments. These tower data indicate an additional attractive coupling rather than the repulsive force consistent with earlier  $g(z)$  observations made in a mineshaft in Australia<sup>4</sup>. However, D. Bartlett and W. Tew (Univ. Colorado) question whether local topography was properly taken into account in the tower experiment, prompting the experimenters carefully to re-examine their data (D. Eckhardt, USAF Geophysical Lab.). In fact the surface gravity measurements were obtained at locations whose mean elevation differed from that of the actual terrain. Correcting for this has reduced the maximum discrepancy between the modelled and observed  $g(z)$  from  $550 \mu\text{gal}$  to  $350 \mu\text{gal}$  ( $1 \text{ gal} = 1 \text{ cm s}^{-2}$ ). Preliminary results from two other tower experiments (T. Niebauer and C. Speake, Univ. Colorado; P. Kasameyer and J. Thomas, Lawrence Livermore National Laboratory) appear so far to support conventional physics. The task of computing newtonian gravity properly is now generally recognized as the most delicate aspect of these experiments, particularly when assigning uncertainties to the prediction.

Gravity measurements performed down the 2,000-m Dye 3 borehole in Greenland were extensively reported last autumn when the preliminary analysis seemed to indicate a departure from the inverse-square law. The experimenters now report that although the borehole  $g(z)$  data (discussed by M. Gorman, Cambridge Univ. and R. Hughes, Los Alamos National Laboratory) disagree with the group's initial newtonian prediction, this discrepancy could well be the result of an intrusion of higher-density

material ( $\Delta\rho \approx 0.3 \text{ g cm}^{-3}$ ) in the rock below the ice cap. The likelihood of such an intrusion is much debated, even by members of the team itself. The group used an optimization code to determine the mass distribution of minimum  $\Delta\rho$  that could account for the  $g(z)$  data while remaining consistent with surface gravity observations. Applying a similar analysis to the gravity data available from the tower and mineshaft measurements (some are proprietary) has led to the claim (R. Parker, Scripps Institution of Oceanography) that the observed  $g(z)$  discrepancies in those experiments can also be attributed to an appropriate local distribution of higher-density material. This suggestion is currently being explored by the experimental teams.

Among the experiments testing the equivalence principle, two<sup>5,6</sup> have produced results consistent with a new material-dependent interaction, but others<sup>7-9</sup> saw no such effects. These initial composition-dependence results were contradictory when interpreted in terms of  $q_s = B$  (see News and Views articles published at the time<sup>10,11</sup>). Efforts have recently been focused on approaches for reconciling the positive observations with the null results, and on extending the search by using apparatus with increased sensitivity.

The suggestion<sup>6,12</sup> that a coupling to  $q_s = B - 2L$  (or isospin) could effect a reconciliation of the composition-dependence results has been closely inspected. This isospin picture amounts to supposing that the neutron and proton are endowed with 'charges' of opposite sign but equal magnitude. Because the Earth's crust (the attractor in the early experiments) is composed mainly of materials (silicon and oxygen) with equal abundances of neutrons and protons, the net  $q_s$  of the various experimental sites would depend on the detailed chemical composition of the local rock.

This hypothesis is readily tested by using either a proton-rich or a neutron-rich substance as the attractor, instead of terrestrial material. One such experiment has been performed by our Eöt-Wash group at the University of Washington, by comparing the interactions of beryllium and aluminium with 1.3 metric tons of lead. We have just published<sup>13</sup> a null result that excludes (at 2 standard deviations) the isospin picture for all scale lengths over which the positive results have been interpreted ( $10 \text{ m} < \lambda < 1,000 \text{ m}$ ).

Preliminary results presented at the meeting from a similar experiment (R. Newman and P. Nelson, Univ. California, Irvine) yielded a comparable upper bound. Another interesting experiment<sup>14</sup> used a torsion balance near a lock on the Snake River in the United States. The quantity of water acting on the balance was varied by the periodic filling and

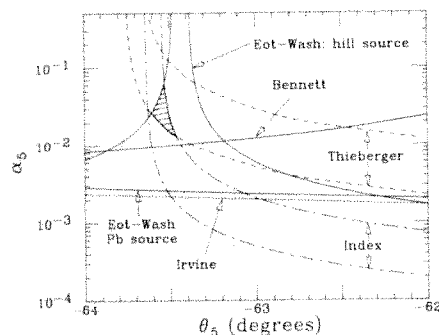


FIG. 1 Constraints on a fifth force coupled to isospin, for  $\lambda = 100 \text{ m}$ . Allowed values of  $\alpha$  ( $\lambda = 100 \text{ m}$ ) are plotted as a function of  $\theta_s$ , where  $q_s = B \cos \theta_s + L \sin \theta_s$ . A coupling to 'isospin' corresponds to  $\theta_s = -63.4^\circ$ . The shaded region shows the area of agreement between the composition-dependence experiments. However, this is now of dubious significance given the lead-attractor results reported by the Eöt-Wash<sup>13</sup> and Irvine (Newman) groups. A slightly less stringent constraint is shown by the lock experiment of Bennett<sup>14</sup>.

\* XXIV Rencontres de Moriond: Tests of Fundamental Laws in Physics Les Arcs, Savoie, France, 21-28 January 1989.

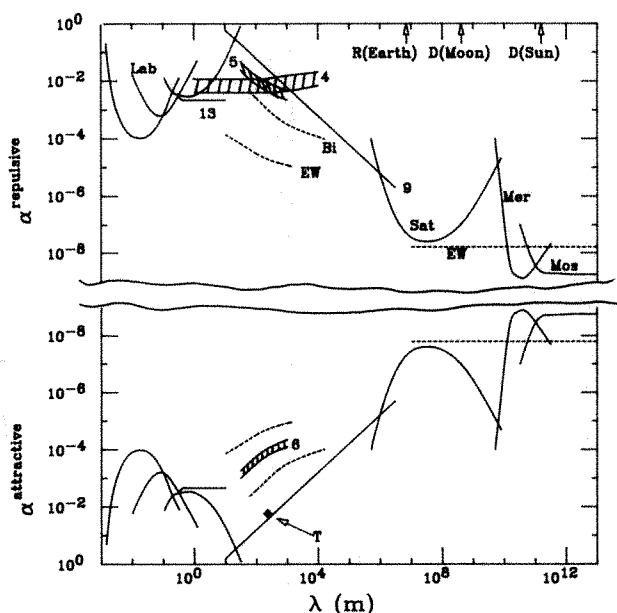


FIG. 2 Constraints on the strength (relative to G) of a Yukawa interaction coupling to  $B$ , for ranges  $\lambda$  between 1 mm and  $10^{13}$  m. The positive results are shown as the shaded regions labelled 4, 5 and 6, in accord with the references. T denotes the best single-Yukawa fit to the revised tower data of Eckhardt *et al.*, who are still in the process of determining uncertainties. For clarity only the most stringent of the many null results are shown, which preclude values of  $\alpha$  greater than the corresponding constraint curves. Bounds that predate the fifth force controversy (see ref. 2) arise from laboratory  $1/r^2$  tests (Lab), from comparisons of the orbits of manmade satellites with that of the moon (Sat), from the precession of Mercury (Mer), and from the equivalence principle experiment performed in Moscow by Braginskii and Panov (Mos). Line 9 is from the freefall experiment of Niebauer *et al.*<sup>9</sup>, and 13 from the Eöt-Wash torsion balance experiment<sup>13</sup>. The dashed segments EW and Bi show the results reported at Moriond by the Eöt-Wash group and by Bizzeti *et al.*

emptying of the lock. The data analysis sought any deflection of the balance that was correlated with water level, and none was found. Results from these isospin experiments are presented in Fig. 1.

New data were presented from two terrestrial-attractor equivalence-principle experiments. A floating-sphere experiment reminiscent of the earlier one by Thieberger<sup>5</sup> was performed on a large-scale escarpment in Italy, by P. G. Bizzeti and collaborators (Univ. Firenze). After a careful search for systematic errors, the experimenters concluded that there was no discernible difference between the hillside's action on the submerged nylon sphere and its influence on the surrounding water. The upper limit on the horizontal acceleration difference of the two materials is  $2.4 \times 10^{-9}$  gal. This null result is particularly significant for  $1.5 \text{ km} < \lambda < 16 \text{ km}$ .

My colleague E. Adelberger presented preliminary hillside results that our team has obtained with a much improved version of our torsion balance. We observe no acceleration difference between beryllium and aluminium at the level of  $4 \times 10^{-11}$  gal, establishing the most stringent limit thus far on a composition-

dependent interaction for  $10 \text{ m} < \lambda < 1.5 \text{ km}$ . The current experimental limits on  $\alpha(\lambda)$  are shown in Fig. 2, with the various results interpreted in terms of  $q_s = B$ .

The initial fifth-force hypothesis of an intermediate-range interaction with  $q_s = B$  is not consistent with experiment. In fact no physically reasonable phenomenological model advanced to date can account for all the data, which is not too surprising given the apparent contradiction in the results.

Applying Occam's razor to the positive observations leads to the suspicion that there are unappreciated sources of systematic error in one or more of these delicate and demanding experiments. It should nevertheless be remembered that only one of the positive observations need be convincingly verified for there to be evidence of new physics. Theorists have shown that a feeble intermediate-range interaction could come about in various ways,

and could be readily accommodated in several pictures of the world beyond the standard model. The possibility that these experiments may provide a window into physics that is well outside the reach of any existing or planned accelerator is sufficient motivation to continue the search. □

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## Digital puppetry

TELEVISION transmission is absurdly inefficient. A TV picture is retransmitted in its entirety about 30 times a second, taking up about 6 megahertz of bandwidth; the new high-definition format will need 30 megahertz. Yet the incremental optical changes could probably fit comfortably into a mere few kilohertz.

So Daedalus's new Electronic Theatre TV system makes no attempt to transmit a picture. Like the human perceptual system, it codes the scene as a physical model: a 'set' or 'backdrop' with a specified appearance, in front of which a 'cast' of objects (furniture, people, cars) stand or move in a specified sequence of positions. From this description, together with defined lighting and camera position, an image-reconstruction routine in the receiver works out what the scene must look like, and displays it.

Transmission bandwidth is utterly minimized. The transmitter downloads its backdrop and cast of characters only once per scene, like a word processor downloading to a printer the font in which a document is to be printed. Thereafter 'stage directions', simple sequences of spatial coordinates and details of changes of appearance, suffice to update the image. Massive real-time computing effort is needed both at transmitter and receiver; indeed, the scheme stretches existing technology to its limits. But since computing speed and power are increasing at a hectic pace, while communication bandwidth (especially geostationary-satellite bandwidth) is ever more scarce and costly, Electronic Theatre should soon be commercially feasible.

The new system will change production methods as well as transmission. Producers and playwrights will increasingly by-pass the camera and studio altogether. They will write a show or a play directly as a script of stage directions — calling up backdrops and casts from stock digitized libraries, manoeuvring them in program form, and studying the resulting visual effect. Viewers, too, will gain. A television set will be able to substitute a local 'font' of items and characters into any broadcast: turning a global soap-opera, for example, into a national, or even a family, story. The chilling uniformity threatened by global TV will be mitigated. Mischievous viewers who want their news read by Mr Gorbachev in drag, say, will also have their wish. And like the live theatre itself, an Electronic Theatre production can be endlessly renewed and re-interpreted. Old dramas will be re-set in different locations, transferred to nostalgic eras, or equipped with exotic characters. Electronic Theatre will not only release bandwidth for thousands of new channels: it will provide the semblance of variety to fill them. David Jones

# Are comet spins primordial?

SIR—Ferrin<sup>1</sup> contends that several comets, including Halley, rotate at rates determined during their primordial accretion. He bases this on the fact that comets satisfy  $L/M \approx M^{2/3}$  ( $L$  is spin angular momentum and  $M$  is mass), a relation approximately fulfilled by virtually all other solar system members. From this, he infers very low densities  $\rho$  for several comets.

Here, I argue that (1) the  $L/M$  relation indicates only that spin periods  $T$  of most Solar System members are about the same; (2) deviations from the constant-spin rule in other small Solar System bodies can be attributed to current processes; (3) the spin rates of asteroids and comets have been significantly altered by collisions and outgassing, respectively, and are thus not primordial; and (4) the  $L/M$  relation, although perhaps useful as a crude guide, is not a reliable indicator of density for a specific object.

For a homogeneous rotating sphere,  $L/M = 0.97M^{2/3}/(TQ^{1/3})$ ; a similar expression is known for triaxial ellipsoids<sup>12</sup>. Thus, if density differences between different celestial bodies can be ignored, the observation that  $L/M \approx M^{2/3}$  implies that rotation periods of Solar System members are roughly the same,  $\sim 8$  h, as originally noted for 70 asteroids and most planets<sup>2</sup>.

Rotation periods of cometary nuclei are poorly known because the classical halo method<sup>3</sup> and precession model<sup>4</sup> are of unproved accuracy. Except for Halley's case, the only reliable values come from recent CCD observations of three cometary nuclei<sup>5</sup> whose rotation periods are 12–22 h, significantly shorter than the 30–50 h periods Ferrin used. As cometary data are so scant, one might ask whether the spins of other small Solar System objects say anything about their beginnings.

The spins of almost 500 asteroids are now known<sup>6</sup>. All but 1–2% have periods within a factor of 3 of the mean,  $\langle T \rangle \sim 9$ –10 h, similar to most planets, a few nontidally despun satellites, and probably not too far from cometary periods. Furthermore, the considerable variations in asteroidal spins with their size<sup>6</sup> belies Ferrin's claim that celestial rotations are fixed primordially. The data show that: (1) large asteroids (radius  $R \geq 62.5$  km) have  $\langle T \rangle \approx 7.5$  h and a maxwellian distribution of spin rates, implying that collisions are important; (2) minor planets with  $R \approx 40$ –50 km spin more slowly ( $\langle T \rangle \approx 11$  h), a fact generally ascribed to angular momentum drain<sup>7</sup>; (3) the smallest asteroids ( $R < 15$  km) rotate faster in the mean ( $\langle T \rangle \approx 8.5$  h) but also have a wider dispersion, especially showing an excess of

slow rotators; they do not obey a maxwellian distribution, perhaps indicating their birth in catastrophic disruption events<sup>8</sup>; (4) spin characteristics also vary with asteroid taxonomy, with M-asteroids, presumed to be metal-rich and relatively dense, rotating fastest<sup>9</sup>.

Small asteroids and comets have had significant angular momentum imparted to them over the aeons. For the former, simple particle-in-a-box calculations<sup>8</sup> find frequent mutual collisions, both erosive and catastrophic, that re-set rotation rates, especially those of the tiniest asteroids. In contrast, comets rarely encounter a compatriot in the spacious Oort cloud nor often meet other projectiles during their brief sojourns within the inner Solar System, so collisions do not appreciably alter their angular momentum. Asymmetric outgassing may however induce comets to twirl faster or slower. The same gas jets that cause the non-gravitational accelerations of cometary orbits produce torques capable of modifying spins. Taking the tangential outgassing reaction force to be  $10^{-6}$  of solar gravity<sup>9</sup> and, for a lower bound on de-spin time, considering this force to have a moment arm  $R$ , spin rates are found to change dramatically over only a few orbits. A similarly swift de-spinning comes from estimating the angular momentum carried away by the sublimated gas. Because expansion velocities in jets are a few hundred  $\text{m s}^{-1}$  whereas typical surface speeds are  $\sim 1$   $\text{m s}^{-1}$ , much less than one per cent of the comet's mass, if lost tangentially to the surface, could remove the body's spin angular momentum. Such a mass is expelled in just a few passes by the Sun.

The rough rule that celestial objects — whether asteroids, comets or pulsars — spin faster when they are denser simply manifests the greater ability of compact objects to hold themselves together as they are spun up. Equatorial surface layers on solid spheres become centrifugally unbound once  $T \leq 3.3$  ( $\rho/g \text{ cm}^{-3})^{-1/2}$  h (refs 2,3,5) while debris on the most distant tips of triaxial bodies will be lost at even slower rates<sup>2</sup>. Loosely bound agglomerates (rubble piles), such as sometimes proposed for comets<sup>11</sup>, flow and elongate at spin periods less than  $\sim 6$  ( $\rho/g \text{ cm}^{-3})^{-1/2}$  h (ref. 12).

Whether one looks at Solar System rotation data in terms of a constant spin-rate law or some universal  $L/M$  plot, one should not take the results too literally. While appearing profound,  $L/M$  relations, which also seem to apply to other classes of astronomical objects, have been dismissed<sup>13</sup> because they merely reflect reasonable upper and lower limits on orbital and/or spin rates, much as I maintain above. Furthermore, data that look so

remarkable on log-log plots over many orders of magnitude in the mass actually show considerable scatter of individual points. As an extreme example, 288 Glauke, an 18.5-km S-object, takes 1,150 h to rotate while the comparable asteroid 321 Florentina spins in 2.87 h; are we to conclude that their densities differ by a factor of 8,000?

These arguments show that the observed rotations of comets and small asteroids are not primordial. Instead, their spins probably result from a partly delimited evolution. Torques, which act sporadically to spin these bodies up or down, cannot produce very rapid rotations because of the centrifugal breakup limit; the end result is that spins are always found somewhere between zero and this limit. Clearly for asteroids, rotation rates do hint at processes of angular momentum transfer. But too little is now known about cometary rotation for cometary origins or properties to be valuably constrained.

On the other hand, it may be that, as the Duchess in *Alice in Wonderland* believed, "If everybody minded their own business, the world would go round a great deal faster than it does."

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## On *Prochlorothrix*

SIR—In his News and Views article<sup>1</sup>, David Penny noted the conflicting results of two comparative biochemical studies of *Prochlorothrix* and other photosynthetic prokaryotes — a study by Turner *et al.*<sup>2</sup> on the 16S ribosomal RNA subunit and a study by Morden and Golden<sup>3</sup> on psbA, a photosystem II-associated protein. Turner *et al.* place *Prochlorothrix* close to *Synechococcus* in a separate line of descent from green chloroplasts, whereas Morden and Golden suggest a closer relationship between green chloroplasts and *Prochlorothrix*. Although these results are inconsistent, it should be noted that both studies<sup>2,3</sup> are a preliminary part of the effort to place the Prochlorophytes on the basis of the limited amount of available



experimental data. The lack of information about possible polymorphisms in natural populations, for example, is an issue which needs to be addressed by further study.

We would like to add that there is an important body of structural data which should be considered before lineage maps are drawn on the sole basis of a few nucleic-acid sequences. The photosynthetic membranes of green chloroplasts have a characteristic, well-studied structural organization, which includes a biochemical segregation of photosystems and other components into stacked (granal) and non-stacked (stromal) membrane regions<sup>5</sup>. Studies on *Prochloron* carried out by others<sup>6</sup> as well as our recent study<sup>7</sup> on *Prochlorothrix* indicate that surprisingly many of the structural details of photosynthetic membrane architecture are identical in green chloroplasts and prochlorophytes. In *Prochlorothrix*, these include membrane appression, an asymmetrical distribution of intramembrane complexes between stacked and non-stacked membranes, the sizes and shapes of particles in membrane fracture faces, and the existence of a tetrameric complex on the membrane inner surface which (in green chloroplasts<sup>8</sup>, at least) is associated with oxygen evolution.

The wealth of structural similarity between prochlorophyte and green chloroplast photosynthetic membranes suggests a much closer relationship between *Prochlorothrix* and *Synechococcus*. It also casts doubt on the ease with which one can suggest, as do Turner *et al.*<sup>2</sup>, that "the acquisition of the ability to synthesize chlorophyll *b* would not seem to be a significant biochemical change". Although the chemical differences between chlorophylls *a* and *b* are indeed minor, the similarities between prochlorophytes and green chloroplasts run much deeper than the aldehyde group of chlorophyll *b*. It is a major pattern of membrane architecture that they have in common, not just the possession of the same chemically modified form of chlorophyll *a*. The independent evolution, in two separate lines of descent, of nearly identical patterns of membrane appression, architecture and photosystem segregation seems, to us, unlikely.

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## Sympatric pest

SIR—Three recent letters<sup>1–3</sup> and an accompanying News and Views article<sup>4</sup> concern the fascinating story of how, in the past 200 years, the native American fly *Rhagoletis pomonella* has apparently moved from its native hawthorne host to become an important pest of commercial apple crops. The assumption is made in all four papers that the clear genetic features characterizing the populations breeding on apples have arisen both sympatrically and very recently, evolving from a resident American population that originally infested only the native hawthorne. But an alternative, if less interesting, explanation for the genetic origin of this pest is also possible.

Before the introduction of the apple, there may have been two or more genetically distinct races of *R. pomonella*, adapted to different hawthorne species or possibly to an endemic native crabapple, such as *Pyrus coronaria*. One such population could have been preadapted for the exploitation of the commercial apple and could then have multiplied, with only minor genetic change, into the abundant pest as we know it today. The rapid sympatric evolution of a host race would not then be a required explanation; the transfer from hawthorne to apple could have been simple colonization of a newly available host.

The hawthorne genus (*Crataegus*) in the north central United States and adjacent regions of Canada consists of over a hundred taxonomically diverse and confusing species belonging to 19 series (species groups)<sup>5</sup>. There are additional varieties, hybrids and/or apomicts. At least 40 recognized species of *Crataegus* occur in the Michigan-Illinois region and more than 20 others are found in the limestone refugia to the south. These latter are ancient highlands that go back to the Permian. They were never covered by glaciers, nor were they ever flooded like the coastal plain or the inland sea. They were the source of most of the colonizers of the recently glaciated areas of Ohio, Michigan, Indiana and Illinois to the north<sup>6</sup>. *R. pomonella* has surely coexisted with many of this truly colossal number of hawthorne species for thousands — or even millions of years, moving south with the plants as the glaciers advanced and then moving back north as the ice retreated.

Must it be assumed that there were no

host races of *R. pomonella* formed during all this time but that a new one was formed in the past 200 years? Sympatric origin of host races is by no means a trivial matter, either for practical pest management or for evolutionary genetics. Accordingly, before accepting the evolution of a distinct host race that arose sympatrically in historic times, further data on the populations that breed on hawthornes must be sought.

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## Our ancestors

SIR—Hominids and the African apes form a well-defined clade, but there is disagreement on the initial divergence of that clade. Most molecular evidence favours the gorilla as the first to diverge, whereas most morphological evidence puts hominids there. The second alternative is mildly supported by evidence from chromosomes<sup>1</sup>. The evidence in each direction is strong and is based on independent and internally complex data, which have been examined by Andrews<sup>2,3</sup> in an excellent review. Later work merely accentuates the problem, which exists primarily because it is taken as axiomatic that the point of divergence was the same for all characters. This need not, however, have been the case.

Perhaps all three groups diverged from the same species, which may have been geographically variable. If so, reassortment of the results of local evolution could produce conflicting apparent phylogenies if one looks at only part of the evidence. As a simple example, the diverse aspects of knuckle-walking and thinner enamel may have begun to evolve in a proto-gorilla subspecies and then been transferred to proto-chimpanzees after the latter mostly separated from proto-hominids.

Such a resolution of the problem is a bit awkward, but on existing evidence it is not as awkward as the alternatives<sup>4</sup>. It has no bearing on classification except to a cladist. Accumulation of further evidence like that now available would support the tritomy (or autotomy?). In contrast, establishment of a long initial divergence time, by fossil or molecular evidence, would make it implausible.

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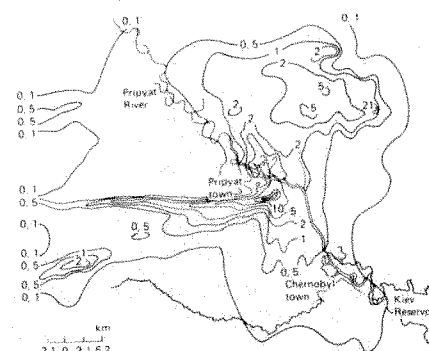
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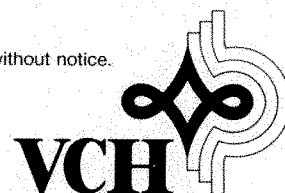
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# Great expectations

Hans Primas

**Beyond the Atom: The Philosophical Thought of Wolfgang Pauli.** By K.V. Laurikainen. Springer-Verlag: 1988. Pp. 234. Pbk DM68, £22.50, \$29.

WOLFGANG Pauli (1900–1958) was a revolutionary genius, a most critical theoretical physicist with profound insight, and a deep thinker. He was an infant prodigy — at the age of 18 he was in full possession of the mathematical and physical knowledge enabling him to do original research on general relativity. At 21 — still a student — he published in the *Encyklopädie der mathematischen Wissenschaften* his masterly review article on relativity theory, which was greatly admired by Einstein himself. Later he became renowned for his fundamental work and brilliant reviews on quantum mechanics and quantum field theory.

Pauli has been called “the living conscience of theoretical physics”, “der fürchterliche Pauli” and “god’s whip” because he attacked every half-truth with pitiless severity. But his criticism was always sound and came to be relied upon. In Pauli’s view, there was no basis for the type of unified field theory pursued by Einstein. But in spite of this and other deep epistemological disagreements, the old Einstein held Pauli in high esteem and called him his spiritual son.

Although Pauli is recognized as one of the leading theoretical physicists of the twentieth century, his long-standing philosophical efforts and penetrating jungian studies are less well known. Only a few of his published articles deal with epistemological problems — the technical papers are remarkably free of philosophical comments. But this state of affairs gives an entirely misleading impression of Pauli’s wide range of philosophical and historical interests, and his far-reaching commitment to jungian thought. Professor Laurikainen’s *Beyond the Atom* — in the main a translation of his Finnish book *Atomien Tuolla Puolen* of 1985 — will, one hopes, change things. The book is based on the extensive but yet unpublished correspondence between Pauli and Markus Fierz, Pauli’s former postdoctoral assistant (1936–1940) who at the time was professor of theoretical physics in Basle.

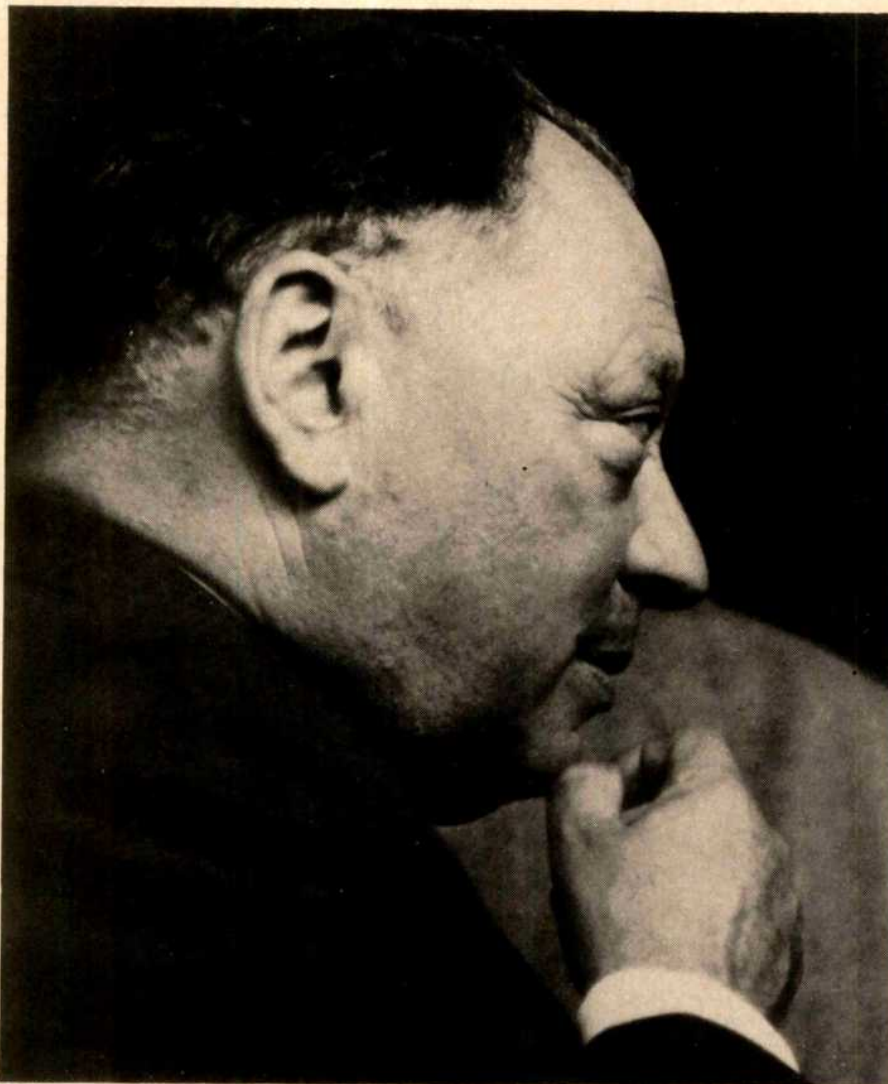
Pauli took up the psychophysical problem by studying the connection between the complex psychology of Carl Gustav Jung and modern physics. In particular, he investigated the archetypal background of physical concepts, and published a remarkable case study on the genesis of scientific theories in the light of the historical controversy between the trinitarian point of departure of Kepler’s astronomy and Fludd’s quaternarian

alchemical viewpoint. Quaternity is taken to be an expression of all concepts of unbroken totality, both in psychology and in physics. The “quaternarian attitude” (an expression often used by Pauli in his letters) is not easy for a classical scientist to grasp. Pauli and Jung discussed the conjecture that the statistical method of natural science could be in a complementary relationship to synchronicity, in the sense that any statistical description excludes synchronistic phenomena. They proposed that the triad of energy, space-time and causality should be complemented by the synchronicity factor to arrive at a “quaternion”, which stands for the unity of being.

In order to express this idea of an unbroken unity, Pauli was looking for a

new conceptual language between the physical and archetypal domains, a language that was neutral with respect to the distinction between physical and psychological phenomena. He conjectured that the alchemistic attempt to create a unitary psychophysical language failed merely because it dealt with a visible reality, and he hoped that the same programme, referring instead to a deeper invisible reality, could be successful. That is, Pauli was striving for nothing less than a coherent and unified conception of the world, in which natural science would be just a part, and which would allow an understanding of matter and psyche as complementary aspects of the same reality — a reality containing both rational and irrational elements. Because the logical structure of complementarity has become transparent within the mathematical formalism of quantum mechanics, it is not so extraordinary that it is just theoretical physicists who have recognized the road sign and have taken up the investigation of complementary relationships outside natural science.

According to Laurikainen, the Pauli-



Wolfgang Pauli — “striving for nothing less than a coherent and unified conception of the world”.



Fierz letters are the best source for the study of Pauli's philosophical views. In his book, he lets Pauli speak for himself in long quotations from the correspondence, unfortunately without Fierz's answers. Laurikainen's selection of material and his analysis of it unavoidably reflect his own tastes and inclinations, but these limitations cannot lessen my regard for his well-thought-out presentation. The most important message of the book is that if we take Pauli's view seriously, we have to re-evaluate fundamental questions in natural science and ponder about the repression of the irrational in Western culture.

With respect to some details, I found myself repeatedly disagreeing with Laurikainen. Fortunately, this disagreement is mainly about questions of present-day quantum mechanics, which was not an issue in the Pauli-Fierz correspondence. Laurikainen believes that Pauli would not have agreed with modern foundational research on quantum theory and the current attempts to generalize quantum theory and to apply it to the meso- and macroscopic domain. To my mind it is wrong to pass such a judgement on the basis of the Pauli-Fierz letters.

In the correspondence, Pauli argued that the general trend of Western culture after the seventeenth century has been dangerously one-sided. He considered the cartesian separation of spirit (*res cogitans*) and matter (*res extensa*) to be misconceived and preferred a vision where spirit and matter are considered as two complementary aspects of reality. Pauli was looking for radically new ideas which went far beyond the limits of quantum theory. According to him, the possibility of free choice of the experimental set-up implies that observations acquire the character of the irrational, unique actuality. But he also stressed that "the psychic state of the observer enters the laws of nature of quantum mechanics no more than those of classical physics", and that "the old question whether the psychic state of the observer possibly may influence the external material evolution of Nature, has no place in contemporary physics". We have to acknowledge that there is a lacuna in the reasonings of present-day physics. Pauli tried to open up new paths, but his programme was very ambitious and remains rather vague, so we cannot implement it in our current theoretical framework. As Pauli himself wrote, the problem is that we must not thereby sacrifice the positive values of the trinitarian attitude. I think that Laurikainen sometimes misconstrues Pauli's vision of a unitary theory as being a comment on present-day quantum mechanics.

**"The most important message of the book is that if we take Pauli's view seriously, we have to re-evaluate fundamental questions in natural science and ponder about the repression of the irrational in Western culture."**

I have one further reservation, also minor. Laurikainen has tried to make Pauli's philosophy understandable to an average academic reader, not only to scientists. Even for the committed reader it is at present the only generally accessible source of Pauli's philosophical correspondence; it is indeed an excellent guide to his views and gives a good first impression of the exchange of ideas with Fierz. But an essay of this nature can never be totally adequate. A full appreciation of Pauli's visions requires a substantial knowledge of quantum mechanics, of the history of ideas and of archetypal psychology. I suspect that most theoretical physicists, psychologists and philosophers who would like to grasp Pauli's ideas stand in need of much more help than they get here. Even Pauli looked for a helping hand — in an unpublished letter (not quoted

by Laurikainen), he expressed his disappointment about the lack of scientific education of Jung's circle, and his hope of finding a discussion partner with profound psychological insight and a good knowledge of mathematics and the natural sciences. So what we urgently need is a complete edition of Pauli's philosophical correspondence with explanatory notes written by various qualified scholars.

Moreover, there is much more pertinent material in the archives. A wealth of supplementary information can be found in Pauli's correspondence with Carl Gustav Jung, Marie-Louise von Franz and Aniela Jaffé, preserved at the ETH in Zurich. There are also related, unpublished manuscripts by Pauli — such as *Die Vorlesung an die fremden Leute*, or his important *Hintergrundphysik* (which is not lost, contrary to Laurikainen's suspicion) — and there is further correspondence, such as that with Heisenberg in the 1950s. Unfortunately, not all of the curators of these papers have allowed them to be published. It would be an intellectual disgrace if the legal heirs of the Pauli estate are allowed to suppress his visions and dreams (which in Pauli's own judgement unquestionably have an objective content), and publish only those letters that are 'relevant' to the history of physics, or that correspond with prevailing scientific fashions. I hope that the appearance of Laurikainen's ground-breaking and thought-provoking book will encourage the publication of a complete and annotated edition of all Pauli's letters and manuscripts in the near future. □

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## The star worm

Paul Sternberg

### The Nematode *Caenorhabditis elegans*.

Edited by William B. Wood and the Community of *C. elegans* Researchers. Cold Spring Harbor Laboratory: 1988. Pp.667. \$94.

IN 1963, Sydney Brenner wrote in a proposal to the Medical Research Council:

Part of the success of molecular genetics was due to the use of extremely simple organisms which could be handled in large numbers: bacteria and bacterial viruses. ... We should like to attack the problem of cellular development in a similar fashion, choosing the simplest possible differentiated organism and subjecting it to the analytical methods of microbial genetics.

Brenner not only chose the nematode *Caenorhabditis elegans* as the organism, but encouraged a generation of scientists to join him; hence, 25 years on, the publication of this first-rate monograph which reviews the work of some 400 scientists. Besides summarizing the basics of *C. elegans* biology, the book presents in digestible form the main accomplishments — determination of the complete cell lineage and of the 'wiring diagram' of the 302-celled nervous system, and the collection of a genetically mapped set of ordered recombinant DNA clones (a genomic map) which is now over 60 per cent complete.

These projects have been carried out by and large at the MRC Laboratory of Molecular Biology, Mecca for *C. elegans* researchers. What then of the unlucky faithful who are unable to make the pilgrimage but who nonetheless want to participate now that the stage is set for a variety of fundamental studies of developmental, neuro- and molecular biology? By reading the book, one can learn much 'worm lore', as well as gain a sense of the excitement in the field.

A scientist starting work on *C. elegans* a decade ago would have had only a handful of publications to read; now one would confront a staggering number of important papers as well as a vast amount of unpublished data. In these days of chasing problems across diverse phyla, researchers often have to grapple unaided with the intricacies of a novel experimental system. Here is help for such people. For example, the often arcane details of the *C. elegans* field (*unc*, *Dpy*, *B.alapaav*, *HSN* and so on) are made accessible to anyone who wants to get to grips with them. The uniformly high quality of the chapters and the extensive cross-referencing not only reflect careful editing, but the cooperative effort of the authors and editor. A great deal of information is presented, however, and some work on the part of the reader is still required.

The application of molecular genetics to muscle, the nervous system and cell lineage is the hallmark of research on *C. elegans*, as is made evident in several cogent chapters. For example, the ability easily to identify and propagate paralysed mutants defective in a myosin heavy chain made possible the first complete sequence of this polypeptide. Identification of over 100 genes controlling various aspects of development relied on many of the characteristics apparent to Brenner in his choice of the worm, especially its small number of cells and short generation time. Yet although the excitement of recent work on sex determination and germline development is amply conveyed, a chapter devoted to the cuticle and molecular genetics of collagen would have been welcome — several genes defined by morphological and behavioural mutants

encode collagens.

The final third of the book is taken up with helpful appendices. The summary of methods is not a complete laboratory manual, but is a good place to start. A 60-page gene list includes invaluable information for the novice (or expert), such as how easy it is to score the phenotypes of the various mutants.

For any biologist, this monograph can serve as an enticement to study a favourite problem in the worm, or as a source of unanswered questions. One hopes that those so encouraged will retain the co-operative spirit, the high standards and the love of this organism that have characterized the field over the past two decades. □

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## The number of the rose

John D. Barrow

**Between Quantum and Cosmos: Studies and Essays in Honor of John Archibald Wheeler.** Edited by W.H. Zurek, A. van der Merwe and W.A. Miller. Princeton University Press: 1988. Pp. 623. \$49.50.

"TIME is God's way of keeping things from happening all at once." Anyone who treasures this amongst their favourite aphorisms is clearly a little out of the ordinary.

John Wheeler is certainly such a person. In honour of his seventy-fifth birthday many of his former students and collaborators cornered six issues of the monthly journal *Foundations of Physics* and filled them with research papers and reviews on subjects to which Wheeler has made fundamental contributions or in which he has a strong interest. Here, these articles are gathered up into a single volume. The result is different both in style and intent from the earlier Wheeler *Festschrift*, *Magic without Magic*, published a decade ago. There are many more contributions, at a much higher technical level, and there are no general biographical articles about Wheeler's scientific career and style of working. Instead there are annotated highlights and characteristic artwork from his most important papers. Yet despite these adornments and the catchy titles of many contributions, this is not a popular book nor even one for scientific outsiders.

The articles fall into five principal categories. The first spans those areas of nuclear physics with a wheelerian pedigree; in particular, the development of the theory of fission (with Niels Bohr), the S-matrix, nuclear models and the role

of nuclear spin-orbit interactions down to the properties of positronium and electrodynamics. The bulk of the book deals with Wheeler's primary interest, gravitation — he was the first to introduce such topical concepts as black holes, wormholes, the Wheeler-de Witt equation of quantum gravity, together with the whole viewpoint of geometrodynamics. The quantum gravity articles in this selection form an interface with those on the interpretation of quantum measurement, where the Everett-Wheeler 'many worlds' interpretation provides the only way of looking at quantum mechanics that makes any sense of the concept of quantum cosmology. The articles by Dicke, d'Espagnat, Ne'eman, Unruh, Braginsky and Khalili, Wothers and Peres all deal with aspects of the quantum measurement problem.

The last and most interesting section of the book contains papers on the concepts of complexity and computation in their most abstract forms. Here there is a long and characteristically insightful discussion of quantum computation by the late Richard Feynman, in which he argues that there are no physical barriers to the reduction of the size of computers to the atomic scale where their mode of operation would become intrinsically quantum mechanical. Landauer and Bennett, two of the foremost contributors to the development of algorithmic complexity as a branch of physics, then each consider the notion of computation and complexity. They show how physics can subject the concept and process of computation to detailed analysis, in order to discover whether the Heisenberg uncertainty principle or the second law of thermodynamics place fundamental limits upon the scope and speed of computation. Bennett provides some discussion of the concept of 'logical depth' as an adjunct to the earlier definition proposed for the complexity of a sequence which used a purely quantita-

tive measure — the shortest program able to generate the sequence — because account must be taken of the difficulty of arriving at the minimal program. Some proposals for achieving this find their way into Bennett's contribution, although the best ideas, which concentrate upon the amount of entropy generated by the program of minimum length, were made after his article was written.

Finally, one should highlight the article by Geroch and Hartle, which describes what it means for a measurable physical quantity to be computable in the sense of Turing, Church and Post. In our mundane experience of physics, non-computable functions have not emerged naturally. It is, though, quite possible for the varieties of differential equation familiar in physics — ordinary differential equations or hyperbolic wave equations — to possess solutions which are non-computable functions of well-posed initial conditions, if one allows the initial behaviour to be not entirely smooth but contain creases and cusps like those found at the point of a cone. Although it is usual for physicists to discount such kinky starting conditions as physically unrealistic, it is not clear that they should do so given the non-continuum nature of classical physics.

Geroch and Hartle discuss a recently discovered example, which arises in quantum cosmology. Here, an observable is found to be equal to a sum of quantities, which must be evaluated for all the compact four-geometries. But the infinite list of these geometries is known to be non-computable. This does not necessarily mean that the observable cannot be computed and predicted — there might be another way that avoids listing the unlistable and so is computable. Nonetheless, it provides an interesting example of how difficult it may prove to answer some of the questions posed in cosmology and particle physics. The ultimate capability of the human brain and of all our computational machines may fall short of that required to rationalize things.

The book makes interesting reading, but it is disappointing in some respects. The articles were originally intended for a journal, and the authors might have written more generally, more speculatively and in a style suited for a wider audience had they set out to compile a book. Aside from the cache of contributions on computation, they show primarily what can be achieved by technical expertise rather than creative imagination. Because John Archibald Wheeler has displayed, and continues to display, the power of the imagination more powerfully than almost any other physicist, that is a pity. But perhaps it shows simply that only John Wheeler can write the articles that John Wheeler writes. □

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## On the beach

Donald J.P. Swift

**The Morphodynamics of the Wadden Sea.** By Jürgen Ehlers. A.A. Balkema: 1988. Pp.397. DM 185, £52.75.

THE Wadden Sea is the intertidal zone of the German Bight of the North Sea. Varying in width from 10 to 50 km, it is an expanse of tidal channels, flats, inlets, flood and ebb deltas, barrier islands and estuaries that extends from Den Helder in the Netherlands to Blåvandshuk in Denmark.

Jürgen Ehlers explains that while mapping the geomorphology of the Wangerooge (map) sheet, "I came to realize the importance of both the major and minor bedforms of the Wadden Sea.... At the same time, my observations led me to suspect that the remodeling of the landscape [was occurring] at a much faster rate than I had previously assumed". With his consciousness thus raised, Ehlers initiated an aerial photographic survey of the Wichter Ee, a tidal inlet between the barrier islands of Norderney and Baltrum, over a 30-day period. The resulting analysis of shoal and megaripple migration forms the core of this book, but there is much more.

After chapters on barrier-island development, recent geomorphological processes, morphodynamic units and historical development, the author rolls up his sleeves and launches into a description, one by one, of the 35 barrier islands of the Wadden Sea and their associated intertidal terrains. This roll call of islands, from Fanø, Mandø, Rømø and Sylt in the North Frisian Islands, through Wangerooge, Spiekerooge, Langeoog and Baltrum in the East Frisian chain, to Terschelling, Griend, Vlieland and Texel in the West Frisian Islands, will for many readers constitute the real strength of the book.

The careful review of historical records is another valuable contribution. Eight centuries of documents allow us to shift from the time scale of fluid dynamical processes at seconds, minutes, days and years into the lower portion of the geological frequency band. Within these eight centuries we can resolve some of the event-dominated fine structure of the most recent phase of the Holocene transgression of the North Sea basin. Important loss of land occurred in the storm surges of the years 1010, 1020, 1041, 1075, 1094, 1102, 1114, 1164 and 1282. The greatest single land loss resulted from the surge of 16 January, 1362, the second Marcellus flood, also known as the *Grosse Mandränke* (great mandrowning). Jade Bay, the Dollart and Harle Bay were enlarged, and in Nordfriesland vast areas of

land disappeared under water, including the legendary Rungholt, east of the present island of Pellworm. A second *Mandränke* occurred on 11 October, 1694. But the main and partially enduring land losses, resulting in the formation of Jade Bay, the Dollart and the Zuider Zee, did not occur as the result of single events, but gradually, through many smaller stages. These land losses were due to a lack of technical infrastructure capable of protecting the vast forelands from the destructive effects of later surges in later decades. Land reclamation occurred, but only through projects that lasted for centuries.

It is important to appreciate this monograph for the fine work of descriptive morphodynamics that it is, and to avoid

viewing it as an indifferently designed work of other purpose. The author's skills lie in the collecting and ordering of information. Chapters that attempt to take an overview, such as those on natural preconditions and barrier-island development, are not altogether successful, although they are always interesting. On the other hand, the relentless procession of maps, aerial photographs and, above all, photograph after photograph at ground level, has a hypnotic effect. Somewhere through the 393 figures, these vistas of misty dunes, beaches and marshes, and of tidal flats extending to the horizon, seep into the unconscious—you have *been* there. □

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## Nature explained

R. McNeill Alexander

**Life's Devices: The Physical World of Animals and Plants.** By Steven Vogel. Princeton University Press: 1988. Pp.367. Hbk \$49.50; pbk \$17.95.

WHY do starfish have five arms? Why don't animals run on wheels? And isn't it intriguing that spider silk has five times the strength of mild steel, and that prairie dogs (rodents, not canines) dig self-ventilating burrows? Here is a brilliant and eccentric book that looks at living things from an engineering point of view, assuming astonishingly little previous knowledge of science on the reader's part.

A summary of one chapter will give the flavour of the whole. Under the same weight of laundry, a sagging clothes line is less likely to break than a tight one. This helps us to understand why there is more tension in the wall of a large pipe than of a smaller one, when both contain fluid at the same pressure: automobile tyres need thicker walls than bicycle tyres (although the latter are inflated to higher pressure), and arteries need thicker walls than blood capillaries. Toy balloons do not inflate uniformly but start with a localized bulge, and blood vessels would be apt to swell similarly were it not for the peculiar properties of their walls. Our lungs need an internal coating of a wetting agent to make their 30 million tiny pockets (alveoli) swell uniformly when we breathe in; which leads to an explanation of why bubbles form against the wall of a beer glass, not in mid-beer.

Professor Vogel suggests two uses for his book: as the basis of a course for non-scientists in liberal studies programmes or, for biologists, as a preparation for deeper biomechanical studies and more advanced reading. *Life's Devices* will be excellent in either role. It is organized by

physical topics: dimensions and scaling principles first, then fluid mechanics, properties of materials, structures and mechanisms. All the mechanics is introduced simply, including tricky topics such as the bending of beams. There are no logarithms or trigonometrical functions, and there is even an appendix as a reminder of how equations work. No background in science is necessary to enjoy the book, only intelligence and the willingness to stop and think.

Here are a great many fascinating scientific stories, including quite a lot that I did not previously know. Too often, though, the explanations are frustratingly short. I doubt whether naïve readers will get a clear understanding of the plastron of aquatic insects, which enables them to breathe under water, from this brief, unillustrated account. I doubt whether they will appreciate the strange properties of slug slime, which enables slugs to crawl while keeping the whole area of the foot perpetually on the ground; as the slug crawls the slime under each part of its foot behaves alternately like a rubbery solid (giving the slug a purchase on the ground) and like a viscous liquid (letting it slide forward). The sparkle of the book depends on its pace, but there were times when it would better have been slowed down.

Professor Vogel is a distinguished biologist who has discovered many revealing things about the flow of water and air over animals and plants. He is also the author of a more advanced biomechanics book, *Life in Moving Fluids*, published by Willard Grant, Boston, in 1981. In an aside half way through his new book he tells us the secret of his success, which is also the book's philosophy: "A good way to make a neat discovery is to begin, not with your favorite organism, but with some physical property or phenomenon and then ask how animals might take advantage of it".

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# A 140,000-year continental climate reconstruction from two European pollen records

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The dramatic and cyclic changes that have characterized the Earth's climate throughout the Quaternary may be illustrated by developments during the last such cycle which took place over the past 140,000 years. Quantitative reconstruction of the continental climate over this period improves the correlation between marine and land records and emphasizes the role of post-temperate forested episodes at the beginning of ice-sheet formation.

IN 1971, Imbrie and Kipp<sup>1</sup> used foraminiferal data obtained from deep-sea cores in palaeoclimate reconstruction. Since then, different multivariate techniques have been used to calculate palaeoclimate parameters from fossil biological data. For continental records, pollen analysis provides the most reliable data<sup>2</sup>. Pollen is deposited as 'pollen rain' which can be characterized by a percentage representation of its components, the so-called 'pollen spectrum'. This provides a reliable reflection of regional vegetation<sup>3</sup>, and hence of regional climate. Past climates can therefore be established from the pollen content of sediments favourable to pollen preservation. Quantitative analysis of such records has until now provided palaeoclimate reconstructions for relatively short periods<sup>4,5</sup>. Here we present the results of applying an improved and simplified method<sup>6</sup>, using pollen-stratigraphic records from La Grande Pile<sup>7</sup> and Les Echets<sup>8</sup>, in eastern France, each containing a sediment sequence for the past 140,000 yr.

The results agree well with the palaeoclimate information derived from marine isotope data and emphasize the role of forested periods before glacial stadials as important episodes of ice-sheet formation. We also show that the arboreal pollen sum, often used by palaeoclimatologists as an index of continental temperature, is inadequate for this purpose.

## Methods

We assume that different climates and their corresponding vegetation types and pollen spectra, which have succeeded each other through time in the study area, can be found today in similar, analogous forms. It follows, therefore, that in order for climates to be represented that are quite different from those at the studied locality today, a very extensive study area is required to provide a wide range of analogues. We therefore first establish representative modern pollen spectra from a large area with wide variations in present-day climate and vegetation; then we define precise climate data for all the sites from which pollen spectra are obtained and identify modern spectra that are most similar to each fossil spectrum. Finally we use modern climate parameters corresponding to the best analogues to infer past climatic conditions.

There are three main problems: The climate requirements of modern plants may be different from those in the past; the range of past climatic conditions may not be fully represented today; and past and present human activity has disturbed the vegeta-

tion/climate, and hence the pollen/climate relationships in the modern pollen data set.

It is unlikely, however, that any major evolutionary changes in trees or herbs (considered here at the genus level) have occurred over the past 140,000 yr. The second problem can be overcome to an extent by using the greatest variety of modern spectra available. We try to minimize anthropogenic effects by using a method for identifying the best analogue spectra; the percentages corresponding to each taxon have been attributed a 'loading factor' which expresses the role it has played at the site throughout the period covered by the reconstruction.

## Data

The modern data, covering Europe, North Africa and Siberia, are represented by 227 spectra selected to represent a wide variety of vegetation types and climates. The 182 spectra described by Guiot<sup>6</sup> are complemented by new data from Morocco, Tunisia, Italy and Corsica, so that a total of 52 taxa could be used.

Annual temperatures and precipitation were interpolated for localities from which the spectra were collected<sup>6</sup>. Among the taxa of fossil spectra, only the 52 taxa used in the modern spectra were considered.

The Grande Pile record (47°44' N, 6°30'14" E, 330 m above sea level) is from three cores<sup>7</sup>: 'Grande Pile 1' for the Holocene (depth: 55–320 cm), 'Grande Pile XIV' for the end of the last glacial (depth: 452–1,193 cm) and 'Grande Pile X' for older periods (depth: 1,243–1,865 cm). We analysed 268 pollen spectra and as 'the miscellaneous and varying content of the pollen spectra from the section 1,170 cm–1,240 cm explains the hypothesis proposed by Gruger (1979) that they include quite a number of rebedded pollen, which deprives them of all botanical and hence climatic significance'<sup>8</sup>, we have neglected this particular section.

The Echets record (45°48'30" N, 4°55'20" E, 267 m above sea level) corresponding to the 39-m core of Les Echets G<sup>8</sup>, yielded 573 spectra (out of the 800 analysed). It was shown<sup>8</sup> that the spectra from the episodes 'Melisey I' and 'Melisey II' were contaminated by reworked arboreal pollen (AP) from earlier temperate deposits, and so for these episodes the mesic AP frequencies were limited to mean values recorded at La Grande Pile for the same periods. As the Les Echets record does not include the Holocene, data for this period are derived from Hières-sur-Amby, 30 km to the south-east but at the same elevation<sup>9</sup>.

## Results

First, we produced a time analysis of each spectra sequence to calculate how each taxon should be weighted as a climate signal. The modern ecological correlation between each taxon and climate cannot be used because of anthropogenic effects. When several well dated contemporaneous sequences are available, information that is common to all of them (mostly of a palaeobioclimatic nature) can be used<sup>6</sup>. In the present case, only the information conveyed from one level to another can be taken into account (Fig. 1a). However, such information is contaminated by noise from the local site peculiarities and (for about

the past 1,000 yr) human activity. Moreover the vegetation at any time is a function not only of the climate at that time but also of previous vegetation and climate.

Now, successive eigenvectors represent different parts of the autocorrelation (Fig. 1a), and therefore only the first eigenvector—in which climate is necessarily dominant—is retained; the others—in which noise is assumed to be dominant—are removed. Moreover, this first eigenvector maximizes autocorrelations and hence the persisting action of climate on vegetation.

The weight attributed to each taxon in the first eigenvector defines its particular role in the biological expression of the climate changes that occurred during the period concerned, and is termed the palaeobioclimatic operator (PBO). When applied to the fossil sequence, it provides a time series which can be considered as the best possible climate profile of vegetation changes.

There is a close correlation between the time series of La Grande Pile and Les Echets (Fig. 2), indicating that the operator, although derived from an autocorrelation analysis made at a single site, has a regional and therefore a climate value. This shows *a posteriori* the effective removal of any noise.

Figure 2 also shows the fluctuation of the AP sum often used by non-botanists as a palynological climate index. Note that the correlation between the AP sum at La Grande Pile and Les Echets, especially in relation to amplitude, is not as clear as that between the PBOs. In addition, comparison between the time series and the AP at each site (correlation coefficient  $r = 0.87$  at La Grande Pile and 0.85 at Les Echets) shows two main differences: the PBO increases later and decreases earlier than the AP sum; and amplitude variations in the PBO are smaller

FIG. 1 The reconstruction method may be summarized as follows: a, The transfer of the climatic information from one level to the other is estimated by the first-order multiple autocorrelation matrix  $A_1$  between the  $m$  taxa, computed on the  $n$  fossil spectra. The element  $(j, l)$  of  $A_1$  is the cross-correlation coefficient between taxon  $j$  and  $l$ :

$$r_{jl} = \frac{1}{n} \left[ \sum_{t=2}^n \frac{f_{jt} - \bar{f}_j}{Sf_j} \frac{f_{lt-1} - \bar{f}_l}{Sf_l} \right]$$

where  $f_{jt}$  represents the frequency (in percentages multiplied by 10) of taxon  $j$  in fossil spectrum  $t$ , the upper-bar represents the mean calculated across the fossil sequence and  $S$  is the standard deviation. As in principal component analysis—with a specific treatment to take into account its asymmetric character— $A_1$  is reduced to a few eigenvectors. The first eigenvector explains ~70% of the sum of squared autocorrelations (72% for La Grande Pile and 69% for Les Echets). The second eigenvector (and the succeeding ones), explaining <10% of this sum, are not retained. The weights  $w_j$  of each taxon in the first eigenvector (called PBO) are used in the second step, and also to provide the time series of the PBO (see Fig. 2):  $b_t = \sum_{j=1}^m w_j (f_{jt} - \bar{f}_j) / Sf_j$ ,  $t = 1, \dots, n$ . b, A weighted euclidian distance operator, integrating the PBO, is used to measure the similarity between fossil and modern pollen spectra and to identify the best-fit analogues for the fossil spectra:  $d_{it}^2 = \sum_{j=1}^m w_j^2 (\ln(f_{jt} + 1) - \ln(f_{ij} + 1))^2$ , where  $t$  is the index of fossil spectra,  $i$  for modern spectra, and  $w_j$  is the PBO loading affected to taxon  $j$ . This set of  $k$  most similar modern spectra is denoted  $(P_{i1}, \dots, P_{ik})$ . However, because the modern data set may not include an exact analogue for each fossil spectrum, the single best-fit modern spectrum may not be appropriate. Instead, we prefer a Monte Carlo simulation of the data, whereby  $k$  spectra are randomly extracted with replacement from the  $k$  selected spectra, and the best-fit spectrum from that group is obtained. This is repeated  $s$  times, providing  $s$  analogues from which confidence intervals are calculated. c, The estimated past climate (analogue climate  $R_t$ ) for a given analogue, indexed by  $k$ , from which the distance is  $d_{ik}$  is given by

$$^0R_t = \left( \sum_{k=1}^s C_k / d_{ik}^2 \right) / \left( \sum_{k=1}^s d_{ik}^{-2} \right)$$

The lower and upper limits of this mean estimate are

$$\begin{aligned} -R_t &= {}^0R_t - \sqrt{\sum_{k=1}^s C_k^2 / d_{ik}^2 / \sum_{k=1}^s d_{ik}^{-2}} - {}^0R_t^2 \\ +R_t &= {}^0R_t + \sqrt{\sum_{k=1}^s C_k^2 / d_{ik}^2 / \sum_{k=1}^s d_{ik}^{-2}} - {}^0R_t^2 \end{aligned}$$

The probability covering the interval  $[-R_t, +R_t]$  is defined by the proportion of modern analogous climate  $C_k$  accounted for within the range of  $s$  values.

than those in the AP during episodes of generally low forest cover.

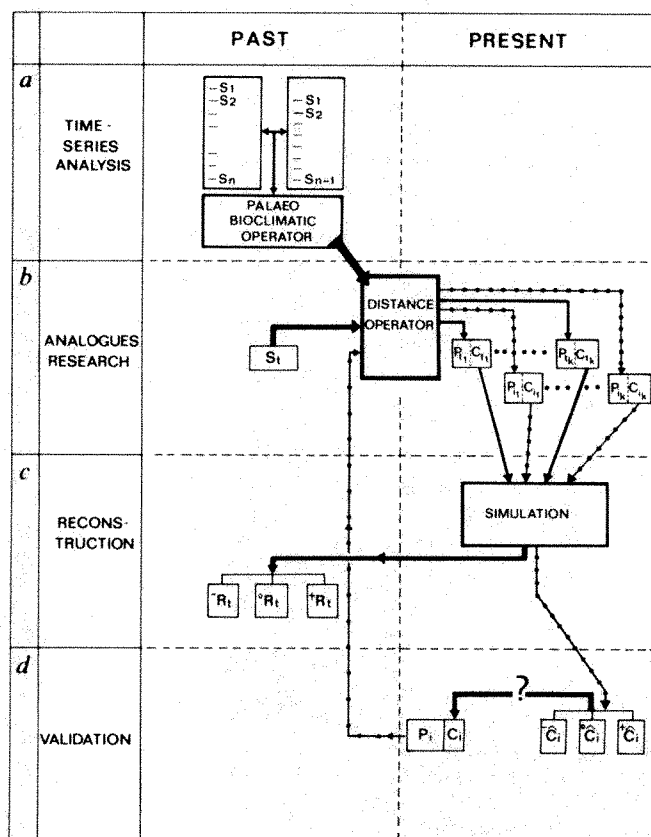
These differences arise because in the AP sum the pollen frequencies of all arboreal taxa are weighted equally whereas in the PBO the percentage of each tree taxon is weighted by its component weighting. For *Pinus* and *Betula*, this weighting may be very different from that of the other trees and close to the coefficient of non-arboreal taxa.

Figure 2 also shows that the variations in the PBO time series correspond more closely than do the AP curves to the oxygen isotope stages defined by Emiliani<sup>10</sup> and Shackleton<sup>11</sup>, represented by the stacked and smoothed record in the SPECMAP timescale<sup>12</sup>.

We next look for analogues. A weighted euclidian distance ('distance operator'), in which the PBO provides a weighting for all pollen frequencies, is used to measure the similarity between ancient and modern spectra, thus enabling us to identify the best analogues for each fossil spectrum.

We then reconstruct palaeoclimate on the basis of the climatic characteristics of the best analogues. A Monte Carlo technique (Fig. 1b) enables us to derive a climate estimate with a confidence level (Fig. 1c) of about 70%. The confidence intervals average 180 mm and 2.1 °C (Table 1). These intervals are asymmetric and slightly larger for Les Echets.

Finally, we investigate both the validity of using a PBO and our method as a whole. We reconstruct climate parameters from modern pollen spectra for which values are known (Fig. 1d). The modern spectra are dealt with in the same manner as the fossil ones, and the reconstructed climatic parameters are compared to actual data (Table 1). The estimates correlate well (0.73 on average). The confidence intervals are slightly narrower than



This interval enables one to appreciate the quality of the analogues. This simulation procedure replaces the more complicated Kalman filtering previously used<sup>6</sup>. d, We use the entire procedure described in b and c, with index  $i$  replacing index  $t$ . The modern climate triplet  $[-C_i, {}^0C_i, +C_i]$  estimated in this way is compared with actual climatic parameters  $C_i$  (see Table 1: modern data column).



TABLE 1 Validation statistics of the reconstructions

		Fossil reconstructions		Modern reconstructions	
		Precip. (mm)	T (°C)	Precip. (mm)	T (°C)
Grande Pile	ME	173	2.1	138	1.8
	*ME	183	1.8	178	1.7
	Cor	—	—	0.77	0.70
Echets	ME	156	2.2	141	1.6
	*ME	198	2.4	164	1.8
	Cor	—	—	0.75	0.71

Cor is the correlation between estimated and actual data, \*ME is the mean upper standard deviation associated to the estimates (\* $R$ –° $R$  in Fig. 1c), ME is the lower standard deviation (° $R$ – $R$  in Fig. 1c). These statistics are calculated on the fossil data and on the modern data. In this last case  $R$  must be replaced by  $C$  (see Fig. 1d).

those computed on fossil data (155 mm and 1.7 °C on average) and have a higher confidence level (>90%). Such good agreement is particularly valuable as the modern estimates are based upon coefficients calculated solely from the fossil data.

The confidence interval mean is larger than that of other reconstructions (13, for example) based upon assumptions of a gaussian distribution and homogeneity between modern and fossil data. As these assumptions are never exactly satisfied, a more appropriate method, including a simulation approach, is used. Such a method provides less optimistic but more realistic confidence intervals. Note, however, that a part of the confidence interval reflects the variability of the calibration data set, so that the cold and dry periods have larger confidence intervals than the warm and/or humid ones. This suggests that the major weakness in our reference data set is a relative lack of cold and dry analogues, which of course it is difficult to compensate for, during the present temperate period.

## Discussion

The reconstructions are obtained as a function of depth. To facilitate comparison with reconstructions from deep-sea cores,

the results are presented on a timescale (Fig. 3). The most important events before 30,000 yr BP are dated on the basis of the widely accepted isotopic chronology<sup>12</sup>, whereas <sup>14</sup>C ages<sup>8,14</sup> are used to date events after 30,000 yr BP (Fig. 2). Intermediate dates are linearly interpolated with steps of 1,000 yr.

We are aware that this procedure does not ensure the independence of our chronology with respect to that of the isotopic stratigraphy. But the succession—if not the chronology—of the climate phases shown by the pollen record is independent, and so are the reconstructed values even though the variation in time is, of course, derived from isotopic records.

The temperature and precipitation curves obtained at both sites agree well. The Holocene and the last interglacial (Eemian) appear clearly as the two warm and humid episodes which followed a complex period marked by significant rises in temperature and precipitation.

A temperature drop, which can be seen during the latter half of the Eemian and is clearly reflected in the vegetation changes, precedes a moisture maximum.

In agreement with botanical evidence, we find that a clear succession of two relatively warm and humid periods occurred after the Eemian. The first, the St-Germain I interstadial, appears as a climatically complex period because at both sites it is interrupted by relatively cold and humid short episodes and the latter half of this period is characterized by a precipitation maximum following a marked fall in temperature.

We found the two periods following the Eemian and the St-Germain I interstadial (the Melisey I and the Melisey II stadials, respectively) to be cold and dry, as expected.

For the second temperate period (the St-Germain II interstadial), we find warm and humid conditions overall, but with a tendency for low temperatures and high precipitation towards the end of the period.

The following period, the Lower Würmian Pleniglacial, was initially humid and cold, then very cold and very dry, as were the 'late Eemian-Melisey I' and the 'late St-Germain I-Melisey II' periods.

The Middle Würm then followed, a dry and cold period but far less severe than the previous stadials. At Les Echets, two

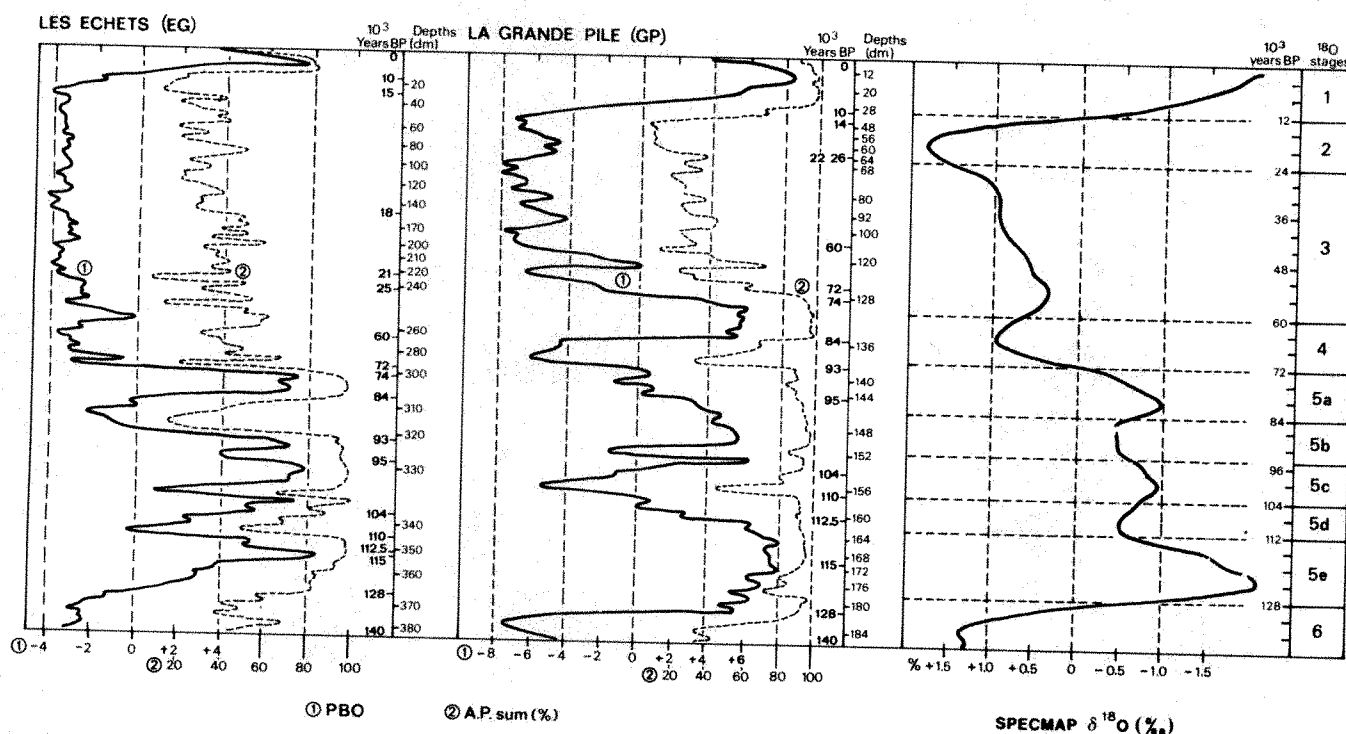


FIG. 2 The palaeobioclimatic operator time series, and the arboreal pollen (AP) sum (%) for Les Echets and La Grande Pile. These series are compared with the stacked, smoothed oxygen isotope record as a function of age using

the SPECMAP scale<sup>12</sup>. The vertical axis is linearly related to the spectra numbers. Estimated ages are given with regard to the depths.

short temperate episodes comprised a rather cool middle phase, and at La Grande Pile we found the same division into two relatively temperate episodes with an intermediate cooler one. These results support the case for a threefold division of the Middle Würm, as suggested by European Atlantic micropalaeontological data<sup>15</sup> and insolation curves<sup>16</sup>. These results, however, need to be verified because they suggest climate parameters which, throughout this period (from ~60,000 to ~30,000 yr BP), are either fluctuating (Les Echets) or poorly marked (La Grande Pile).

Because of differences in the sediments, it is difficult to compare results for the two sites for the Upper Pleniglacial. Climate, during this period, was chiefly characterized by continuous low temperatures, no such equivalent being found since the beginning of the Eemian.

Low-amplitude oscillations, mainly in relation to moisture, are recorded repeatedly, especially at Les Echets, but on the strength of botanical and sedimentological data, they are believed to result from local fluctuations rather than regional general climate variations<sup>17</sup>.

The Younger Dryas is not present in the available record of La Grande Pile and is poorly marked in the Les Echets/Hière-sur-Amby succession.

### Implications

If one agrees with Ruddiman and McIntyre<sup>18</sup> that an accumulation of continental ice implies a cold and humid continent, as opposed to a hot Atlantic Ocean between latitudes 50° and

60° N, the results presented here suggest that there were three major ice-accretion periods in Europe.

The first corresponds to the very humid and markedly cold climate of the final part of the Eemian, a prelude to the even colder and dry Melisey I stadial. The second is the end of the St-Germain I interstadial (very humid but moderately cold) which was succeeded by the cold and dry Melisey II stadial. The third ice-accretion period corresponds to the end of St-Germain II interstadial and to the beginning (markedly cold but moderately humid) of the Lower Pleniglacial, before the second very cold, dry part of this major stadial.

The main ice-accretion period, which brought about an 'inception' period<sup>11</sup> (increase of global ice above modern values), started before 110,000 yr BP, the date cited as the end of the Eemian Interglacial<sup>12</sup>. Note that the date 115,000 yr BP corresponds to minimal insolation in June, July and August at 60° N (ref. 16). A comparison of the Earth's orbital parameters at 115,000 yr BP with those of 125,000 yr BP suggests a cooling of the soil and an increase of soil moisture<sup>19</sup> in regions situated between the Mediterranean Sea and Siberia.

Isotopic and pollen analyses of an eastern Atlantic core have shown that the fall in the oxygen isotope curve based on benthic foraminifera—the beginning of the first ice-growth phase somewhere within polar latitudes—was contemporaneous with the expansion of *Carpinus* in Europe<sup>20</sup>. However, our results suggest that in Europe, the first glacial accretion began only after the *Abies* forestation episode, which followed the *Carpinus* forestation episode. This accretion, evident in foraminifera  $\delta^{18}\text{O}$

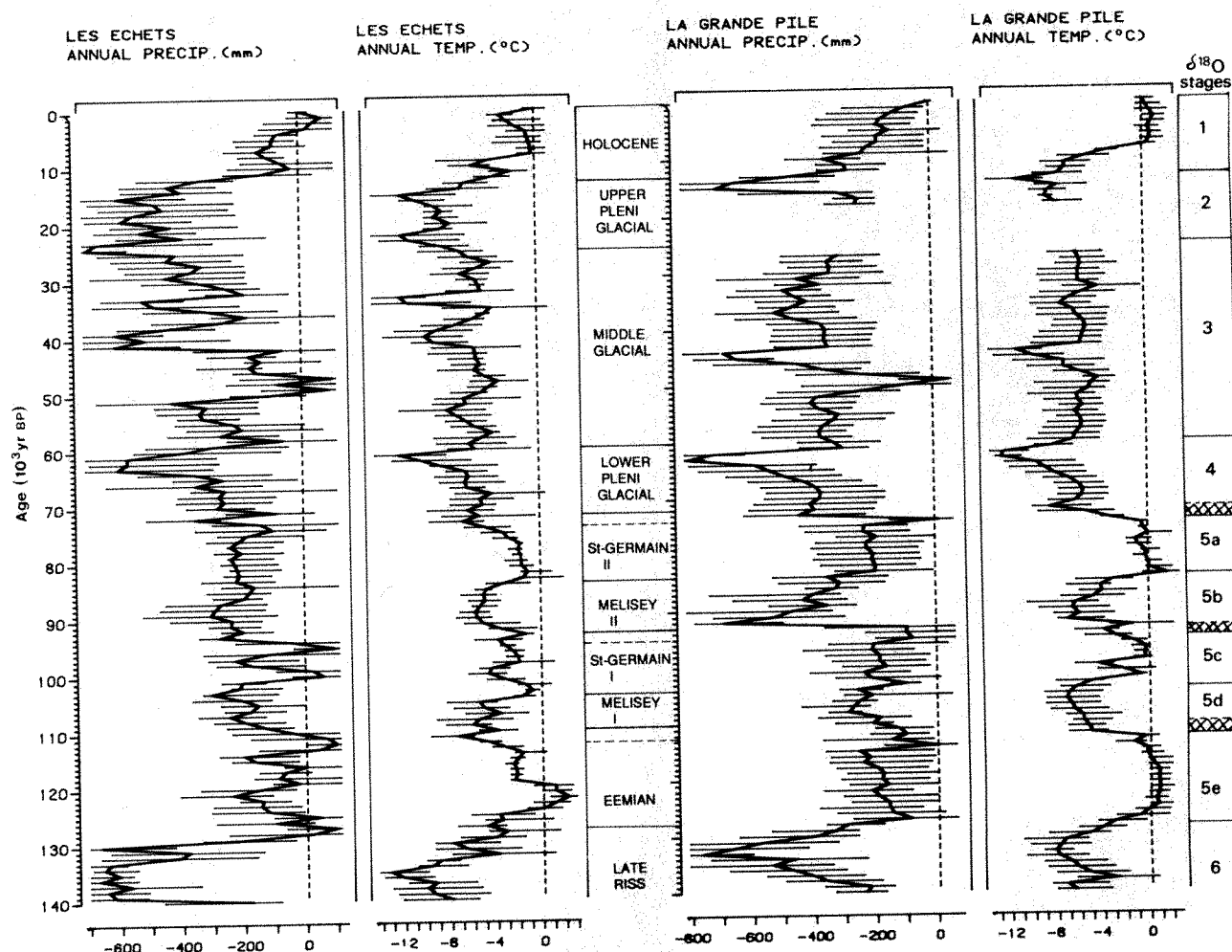


FIG. 3 Reconstruction of variations in annual total precipitation and mean temperature, expressed as deviations from the modern values (1,080 mm and 9.5 °C for La Grande Pile, 800 mm and 11 °C for Les Echets). The error

bars are computed by simulation. The vertical axis is obtained by lines interpolation from the dates indicated in Fig. 2.

data, should therefore be located in the north-west Atlantic. Changes in insolation that occurred around 115,000 yr BP and which are considered to account for this event<sup>20</sup> would then have brought about the glacial accretion on the European side of the Atlantic, with a substantial delay, as has been suggested previously<sup>28</sup>. As our chronology depends on the isotopic chronology, however, we cannot say how long this delay was.

It is generally advocated (for example, refs 14 and 20) that marine isotopic stages or sub-stages characterized by low  $\delta^{18}\text{O}$  values correspond exactly with stadial or pleniglacial continental episodes. Our reconstruction shows, during the first part of the last climatic cycle, well characterized transitions between periods of either low oceanic  $\delta^{18}\text{O}$  values (interglacial or interstadial climatic optimums) or high ones (stadials or pleniglacial). As a result, the lower limit of the continental equivalent of isotopic sub-stages 5d and 5b and of stage 4 should be situated within transition periods and not correlated exclusively and directly with the Melisey I and Melisey II stadials or the Lower Pleniglacial, so that our results are more consistent with the interpretation of the marine isotopic stratigraphy concerning the duration of substages 5d and 5b and of the stage 4 than is the previous interpretation of the continental climatic record<sup>14,20</sup>. It is

noteworthy that the transition periods, which play a major part in initiating ice-sheet formation, are forested periods. They correspond to *Picea*, *Pinus* and *Betula* forests, which mark the latter part of temperate episodes that follow the warm climate optimum; such forestation episodes are therefore often called 'post-temperate'.

The palaeobioclimatic operator appears to be the best botanical indicator of climate change, and should be preferred to the AP sum, which is unreliable, particularly for estimating palaeotemperatures. However, our reconstruction clearly suggests quite temperate conditions for the St-Germain I and St-Germain II interglacials, conditions almost similar to those of the present day, especially in relation to temperature. Such temperate phases are not recorded in the Antarctic ice cores<sup>22</sup>, in Pacific Ocean records<sup>23</sup>, or in Atlantic Ocean deep-water temperature estimates<sup>21</sup>. More surprisingly, neither are they found in records from northern Europe<sup>24-26</sup>. This might indicate a steeper thermal gradient for these periods than for today. By applying our method to pollen sequences from Padul (Andalusia)<sup>27</sup> on the one hand and to successions which contain records of these interstadials in northern Europe on the other, it should be possible to test this hypothesis. □

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# The *Caenorhabditis elegans* heterochronic gene *lin-14* encodes a nuclear protein that forms a temporal developmental switch

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During wild-type development, a protein product of the *Caenorhabditis elegans* heterochronic gene *lin-14* is localized to nuclei of specific somatic cells in embryos and early larvae, but is absent in late larvae and adult soma. Gain-of-function *lin-14* mutations cause the level of *lin-14* protein to remain high throughout development, resulting in developmental reiterations of early cell lineages. The normal down-regulation of the *lin-14* nuclear protein level encodes a temporal switch between early and late cell fates.

DURING the development of multicellular organisms, genes specify the proper developmental fates of cells in particular spatial domains<sup>1,2</sup> or particular cell lineages<sup>3,4</sup>; mutations in such genes transform the fates of cells into those normally found at different positions or lineages. In *C. elegans*, the temporal pattern of the cell types generated during development is explicitly controlled by heterochronic genes in an analogous manner<sup>5-7</sup>. Heterochronic mutations cause particular cells in various cell lineages and tissues to adopt fates during post-embryonic development that are normally associated with cells at earlier or later stages of development. An analysis of mutations in the heterochronic gene *lin-14* has indicated that this gene plays a central role in controlling the temporal pattern of the *C. elegans* post-embryonic cell lineage<sup>6,7</sup>. Loss-of-function *lin-14* alleles cause the precocious appearance, during early larval



stages, of cell lineages that would normally be observed in descendent cells one or two larval stages later. Conversely, gain-of-function *lin-14* alleles affect the same cell lineages but cause the opposite transformations in cell fate; thus, the early cell lineages are normal, but later cells reiterate the early cell lineages normally associated with their ancestor cells. These results indicated that relatively high *lin-14* gene activity early in development is normally reduced later in development, and that this change in *lin-14* gene activity controls a switch from early to late cell fates in all of the cells affected by *lin-14* mutations<sup>7</sup>.

The *lin-14* gene has now been cloned using a restriction fragment length polymorphism (RFLP) mapping approach<sup>8</sup>. Here we use antibodies to the *lin-14* protein to observe the cellular and subcellular location of the *lin-14* protein and the temporal regulation of its accumulation during wild-type and *lin-14* mutant development. We find that the *lin-14* protein is localized in the nucleus and that its progressive elimination during *C. elegans* development controls the normal temporal sequence of cell fates mediated by the *lin-14* gene.

### *Lin-14* protein is nuclear and temporally regulated

To overproduce the *lin-14* gene product in *Escherichia coli*, we fused the *lacZ* gene of plasmid pUR290 (ref. 9) in phase with an open reading frame from a *lin-14* cDNA clone, number 557. The *lacZ-lin-14* fusion protein was partially purified by preparative SDS gel electrophoresis and used to immunize rabbits. The resulting antisera were affinity-purified using a *lacZ-lin-14*

fusion protein column. Figure 1a shows that this antibody preparation is *lin-14*-specific.

To detect the *lin-14* protein in *C. elegans*, cell extracts were prepared from staged cultures of *C. elegans* and analysed by immunoblotting using the anti-*lin-14* antibodies. A protein of relative molecular mass ( $M_r$ ) 70,000 (70K) was detected that is most abundant in early larval stage animals but not in later stages (Fig. 1b). This 70K protein is absent in two *lin-14* null mutants (Fig. 1c), showing that it corresponds to (or, less probably, is positively regulated by) the *lin-14* gene product.

We used the affinity-purified anti-*lin-14* antibodies to detect the *lin-14* protein in whole-mount fixed specimens of wild-type *C. elegans* by indirect immunofluorescence staining, and found that the anti-*lin-14* antibodies bound antigen in specific somatic nuclei of late embryos and early larvae (Fig. 2). No immunofluorescence in somatic cells was detected in strains bearing the loss-of-function *lin-14* alleles *n536n540* and *n355n726*, which by genetic criteria behave as null alleles<sup>7</sup> (data not shown), showing that in wild-type specimens the anti-*lin-14* antibody binds the *lin-14* protein itself (or, less probably, a gene product positively regulated by *lin-14*). Because both the 70K protein detected on the immunoblots and the somatic nuclear *lin-14* staining disappear in these *lin-14* mutant strains, the somatic nuclear staining probably corresponds to this 70K *lin-14* protein.

Mutations in *lin-14* change the developmental fates of certain post-embryonic blast cells<sup>6,7</sup> (Fig. 3). By determining precisely which cells stain with the anti-*lin-14* antibody, we correlated

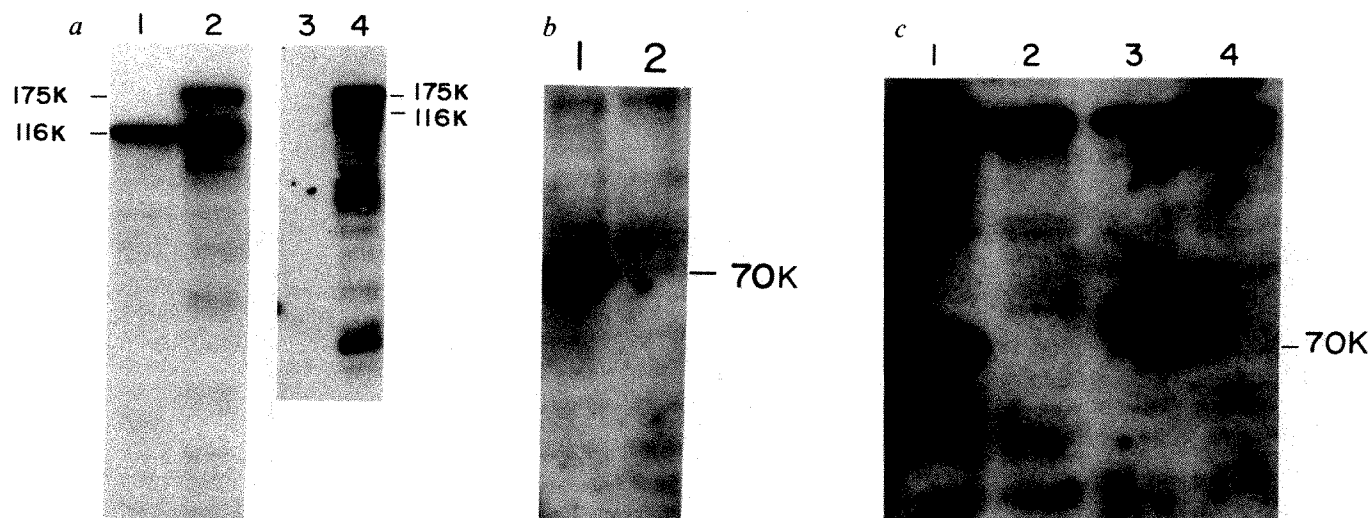


FIG. 1 a, Specificity of antibodies to *lin-14* on immunoblots. Lanes 1 and 3, protein extracts of IPTG-induced JM101/pUR290 cells. Lanes 2 and 4, protein extracts of IPTG-induced JM101/pUR290-cDNA557 cells. Lanes 1 and 2 were probed with sera partially purified by affinity chromatography with protein extracts from *E. coli* expressing the *lacZ-lin-14* fusion gene. The partially-purified sera recognizes both *lacZ* (lane 1) and *lacZ-lin-14* (lane 2) epitopes. Lanes 3 and 4 were probed with further purified sera from which anti-*lacZ* and anti-*E. coli* antibodies had been removed by affinity chromatography with protein extract from *E. coli* expressing *lacZ*. This sera detects only the *lacZ-lin-14* fusion protein (lane 4) (Lower  $M_r$  bands are presumably degradation products of this fusion protein that retain *lin-14* epitopes.) This sera was used for all immunological detections of *lin-14* protein in *C. elegans* protein samples and fixed specimens. The  $M_r$  of the *lacZ-lin-14* fusion protein is 175 K. b, Detection of the *lin-14* protein in extracts of wild-type *C. elegans*. The sera specific for *lin-14* was used to probe a western blot of protein samples isolated from staged *C. elegans* strains: lane 1, wild-type (N2) larval stage 1; lane 2, wild type (N2) larval stage 2. c, Lack of *lin-14* protein in two *lin-14* null mutants: lane 1, wild-type (N2) larval stage 1; lane 2, *lin-14*(*n536n540*) larval stage 1; lane 3, *lin-14*(*n355n726*) larval stage 1; lane 4, *lin-14*(*n355n726*) larval stage 3. The large protein on this blot was detected in both wild-type and *lin-14* mutants with some antibody preparations. Because it is not affected in these *lin-14* mutants, it may correspond to a cross-reacting protein.

METHODS. A cDNA library from *C. elegans* mixed-stage mRNA was constructed in  $\lambda$ gt10 using standard protocols<sup>17</sup> and screened with *lin-14* genomic clone probes. The *lacZ-lin-14* fusion gene was constructed by ligating *Sal*I-digested pUR290<sup>9</sup> DNA to *Xho*I-digested *lin-14* cDNA557 DNA using standard techniques. The antibody was generated by immunizing New Zealand White rabbits with 500  $\mu$ g of *lacZ-lin-14* fusion protein per rabbit at 20 sites. To affinity-purify the sera, total protein extracts from JM101/pUR290Sal-*Xho*I-cDNA557 and from JM101/pUR290 were each bound to derivatized beads (Affigel-10, BioRad)<sup>18</sup>. The sera was first adsorbed to the *lacZ-lin-14* affinity column, and subsequently, anti-*lacZ* and anti-*E. coli* protein antibodies were removed by exhaustive adsorption of this sera with the JM101/pUR290 protein extract affinity column. Western blots to *E. coli* extracts containing *lacZ* or *lacZ-lin-14* protein<sup>19</sup> used a 500-fold or 1,000-fold dilution of the affinity purified anti-*lin-14* antibody. Antibodies were diluted 50-fold or 100-fold for western blots to *C. elegans* extracts. Bound antibodies were detected using <sup>125</sup>I-Staphylococcus aureus protein A (New England Nuclear) at 5  $\mu$ Ci ml<sup>-1</sup> (ref. 19).

the temporal and cellular pattern of *lin-14* protein accumulation with the cell lineages affected by mutations in the *lin-14* gene and with the known developmental fates of all those cells.

The *lin-14* protein was first observed in embryos at about 7 hours after fertilization or about half-way through embryogenesis, after which time most of the embryonic cells had been generated, and cell differentiation and morphogenesis were taking place<sup>10</sup>. At this stage, the most intense staining was in intestinal and hypodermal nuclei (Fig. 2a). In later stage embryos, at about 9 hours after fertilization, additional weak neuronal staining was observed (data not shown). No lineage defects before larval stage one (L1) have been observed in *lin-14* mutant strains, and the temperature-sensitive period for *lin-14* lineage defects occurs after hatching<sup>7</sup>, indicating that the accumulation of the *lin-14* protein detected in these embryos is not yet functional.

The most intense staining that we observed at any stage was in nuclei of late embryos just before hatching, and in newly hatched L1 animals. Significantly, only nuclear staining was observed at these stages (Fig. 2b, c). In the newly hatched L1 animal, the *lin-14* protein was present in the nuclei of most of the post-embryonic blast cells; intense nuclear staining was observed in the hypodermal blast cells H1, H2, V1-V6, and T (Figs 2c and 3) and in all of the intestinal (E) cells (Fig. 2b), and weaker staining was observed in both neuroblasts Q1 and Q2, in the mesoblast M cell, and in the P cells. No staining was observed in the somatic or germ line gonadal blast cell nuclei (Z1 to Z4) at this stage.

During the L1 stage, the hypodermal blast cells H1, H2, V1-V6, T, and intestinal cells, E, all divide<sup>11</sup>. The nuclei of these progeny cells also stain with the anti-*lin-14* antibody during the mid-L1 stage. At this stage the P-cell nuclei migrate into the ventral cord and the cells divide<sup>11</sup>. The *lin-14* staining in the P-cell nuclei fades before their migration into the ventral cord but reappears later in some of their progeny cells (see below).

The intestinal (E), hypodermal (H1, H2, V1-V6, T), neuroblast Q1 and Q2, mesoblast (M), and P-cell lineages are all affected by *lin-14* mutations; loss-of-function *lin-14* mutations cause these cells to precociously express late stage patterns of cell lineages<sup>6</sup>. Wild-type blast cells accumulate the *lin-14* protein before the first cell lineage defects can be observed during the L1 stage in *lin-14* loss-of-function mutants and before the L1 temperature-sensitive period of some *lin-14* mutations<sup>7</sup>. The gonadal blast-cell lineages are not affected by *lin-14* mutations<sup>6</sup> and do not accumulate the *lin-14* protein. Thus, all post-embryonic blast cells affected by *lin-14* mutations also accumulate the *lin-14* protein at the expected time, and those post-embryonic blast cells not affected by *lin-14* mutations do not accumulate the *lin-14* protein.

The nuclei of many cells that do not divide post-embryonically also accumulate the *lin-14* protein during the L1 stage. The embryo-derived nuclei in the hypodermal syncytial cell hyp7, ABArpppapa, ABplaapppp, Cpaaaa, Cpaapa, Cpaapp, Cpapaa, all accumulate levels of the *lin-14* protein similar to those of the hypodermal blast cells (Figs 2c and 3). Terminally differentiated nuclei from embryonic body muscle also accumulate the *lin-14* protein. Nuclei of many but not all neuronal cells stain with the anti-*lin-14* antibodies. For example, the neurons BDU, ALM and CAN accumulate the *lin-14* protein but the HSN neuron does not (Fig. 2c, data not shown). All of the embryonically generated ventral-cord neurons, and some but not all of the neurons of the nerve ring and posterior ganglion accumulate the *lin-14* protein in their nuclei during the L1 stage (Fig. 2c, d, e).

These embryonic hypodermal, muscle, and neuronal cells are not affected by *lin-14* mutations but nevertheless accumulate the *lin-14* protein<sup>6</sup>. In the characterization of the *lin-14* mutants, changes in the patterns of cell division and differentiation of only blast cells, which divide post-embryonically, were scored; the effect of *lin-14* mutations on non-dividing cells was not

assessed. It is not known, therefore, whether the *lin-14* expression that we observe in these differentiated cells is in fact functional.

Late in the L1 stage, the *lin-14* protein staining of all nuclei except the neuronal nuclei is much weaker. At this stage more neurons of the nerve ring and posterior ganglion stain than at earlier stages (data not shown). Thus, in the hypodermal and intestinal cell lineages, *lin-14* protein levels peak during early L1 and fade entirely by L2. In the many neuronal cells, *lin-14* protein levels peak during mid to late L1 and fade by L2. By L2 and in subsequent larval stages, most animals showed no *lin-14* staining at all (Fig. 2f, g). The disappearance of the *lin-14* protein during the L1 stage from the nuclei of the post-embryonic blast cells correlates well with the mid to late L1 temperature-sensitive period for *lin-14* mutations<sup>7</sup>.

The temporal regulation of *lin-14* protein accumulation is displaced from this L1 peak in some cell lineages. The Pn.p cells, which do not divide until the L3 stage, do not accumulate the *lin-14* protein until that stage when they show very weak staining before the division (data not shown). This staining fades by early L4. The accumulation of the *lin-14* protein in the Pn.p cells during L3 correlates with the precocious Pn.p cell divisions, abnormal Pn.p-derived cell lineages, and resulting abnormal morphogenesis of vulval structures in *lin-14* mutants<sup>6</sup>. The temperature-sensitive period for this defect has not been determined.

In occasional L2 and L3 stage animals, weak *lin-14* protein staining in hypodermal, neuronal, and intestinal cells was observed in nuclei and cytoplasm, as if it was being slowly degraded or not efficiently transported to the nucleus. Patches of *lin-14* staining in hypodermal or intestinal nuclei was only rarely observed in very old adults (data not shown). Such sporadic accumulation of the *lin-14* protein indicates that the gene may be re-expressed under some unknown physiological conditions.

In most adults, *lin-14* immunostaining reappears only in the mature oocyte nuclei of hermaphrodites (Fig. 2h), at meiotic prophase I when the chromosomes are condensed as shown by DAPI staining (Fig. 2i). This oocyte expression of *lin-14* was not predicted by genetic analysis; no *lin-14* maternal effect has been detected (V. Ambros, personal communication). The oocyte nuclear immunofluorescence, however, remained in the *lin-14* loss-of-function mutant strains *n536n540* and *n355n726* (data not shown), indicating that these are not true null mutations for all tissues that express *lin-14*, or that the oocyte-specific antigen detected is not in fact a product of the *lin-14* gene, but rather an antigenically related gene product. The oocyte *lin-14* nuclear staining disappears after fertilization; presumably it is either degraded or greatly diluted during nuclear division. No staining is visible in early embryos.

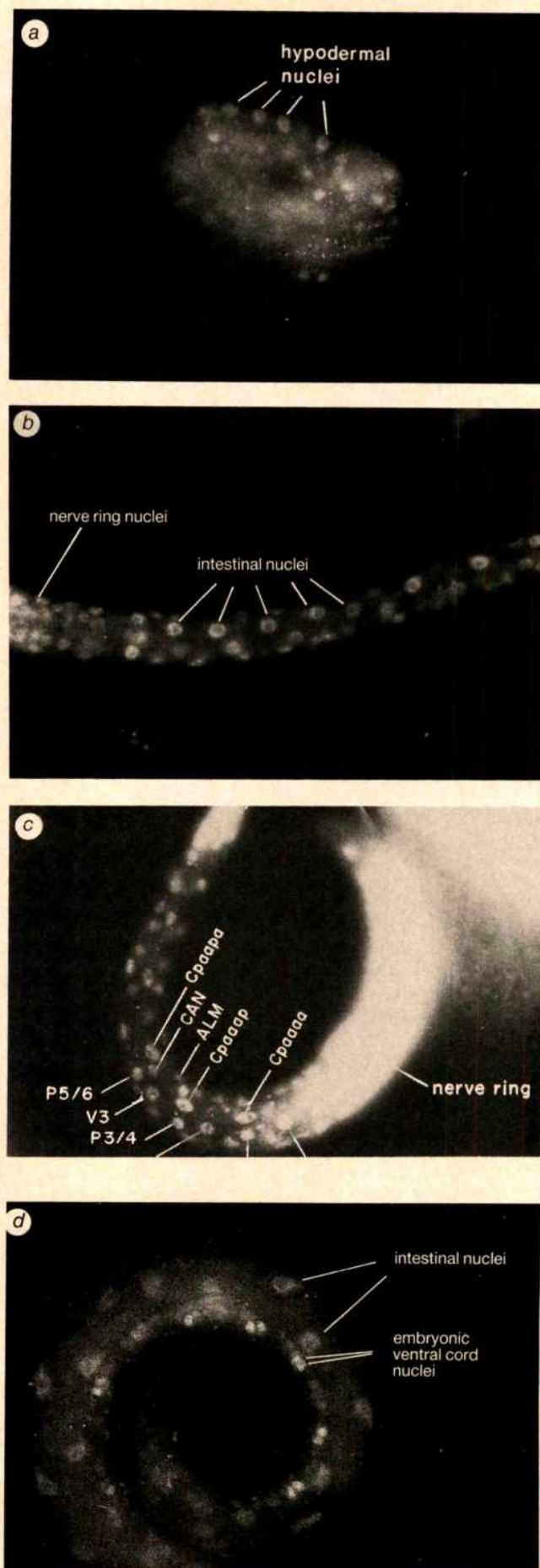
### *Lin-14* mutations

Gain-of-function *lin-14* mutations cause reiteration of early larval cell lineages at late larval and adult stages<sup>6,7</sup>. The embryonic and L1 stage staining in these mutants was equivalent to that seen in the wild-type in its intensity, nuclear localization, and cellular distribution (data not shown). Unlike the wild-type, however, these mutants showed high levels of the *lin-14* protein in many nuclei during larval stages 2, 3 and 4 and in adults (Fig. 2j, k, l). Protein staining was also observed in hypodermal, intestinal, muscle and neuronal nuclei in these late larvae and adults. All of the post-embryonic blast cells known to be affected by gain-of-function *lin-14* mutations inappropriately accumulate the *lin-14* protein at these late stages. For example, during the L3 stage, the hypodermal nuclei V4.ppp, V5.pppp and Tapapap, which in these mutants will divide like the wild-type cells V4, V5 and T, respectively<sup>6</sup>, accumulate the *lin-14* protein (Fig. 2j). The abnormal presence of the *lin-14* protein in these cells seems to change their fates into those of V4, V5 and T, respectively. Similarly, in the adult stage, the cell V4.pppp divides inappropriately in these mutants<sup>6</sup> and also accumulates

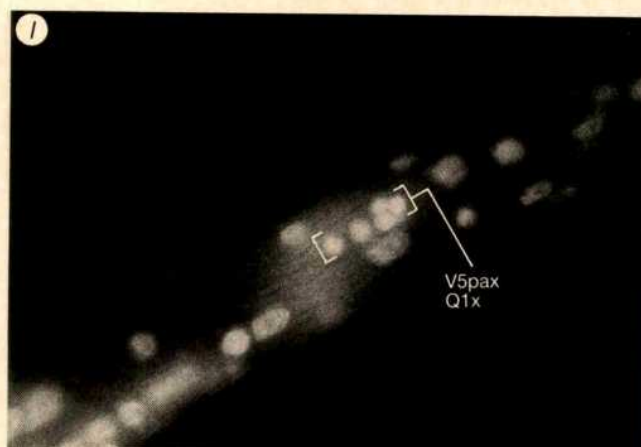
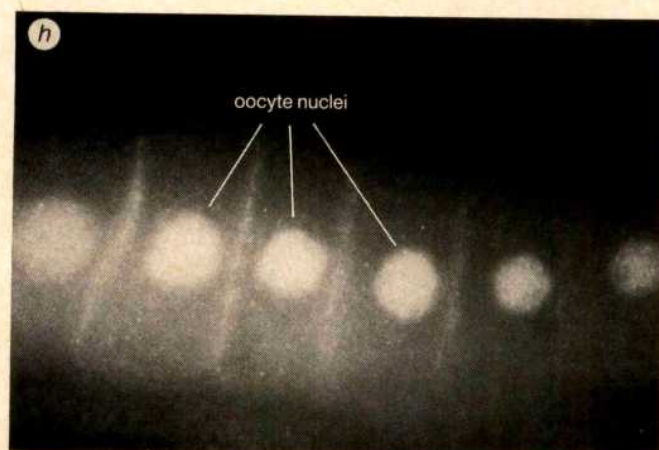
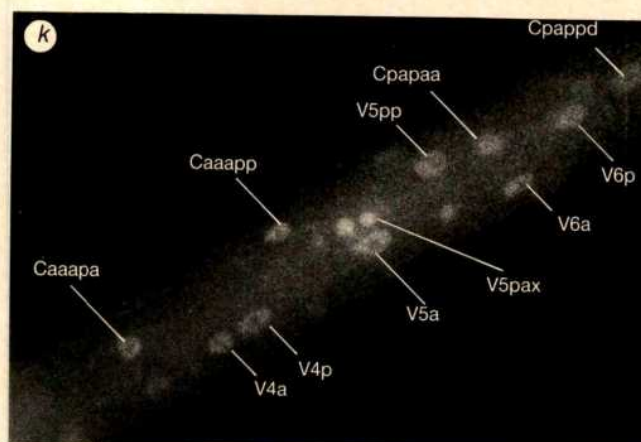
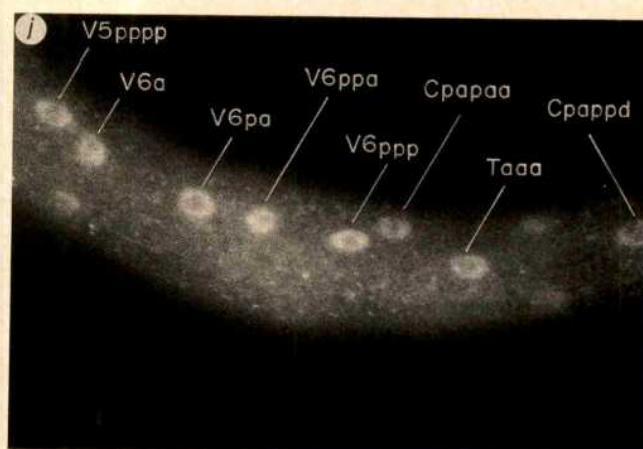
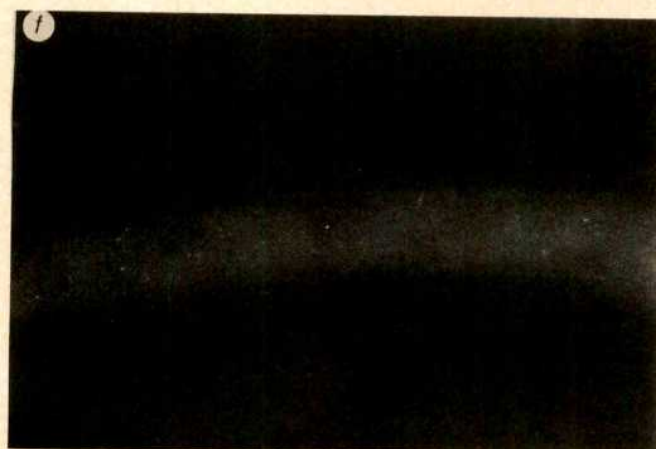
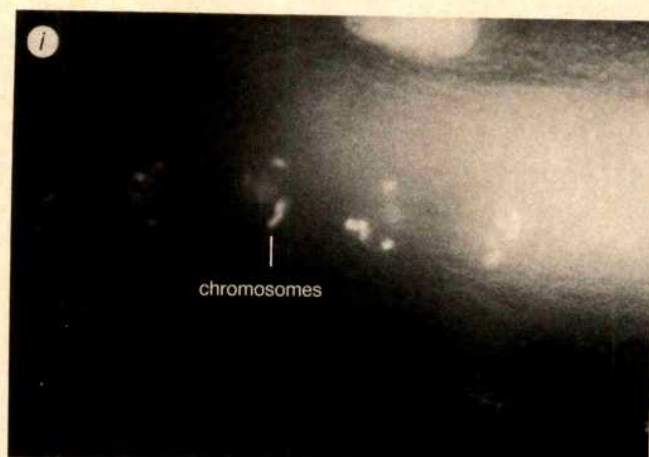
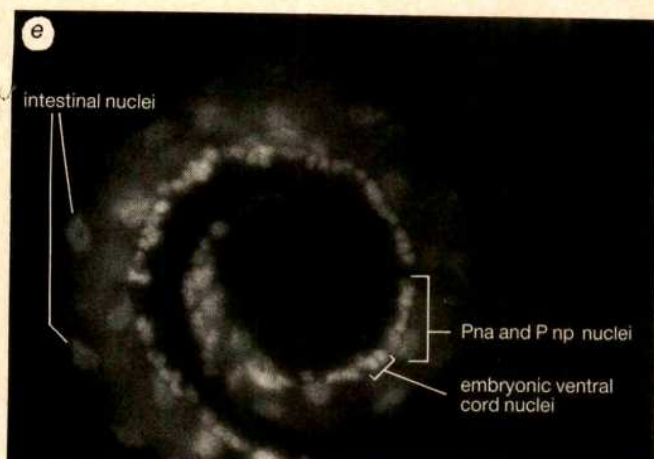


**FIG. 2** Immunodetection *in situ* of the *lin-14* protein. Specimens were fixed, made permeable and incubated with affinity-purified rabbit anti-*lin-14* antibody followed by secondary fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit antibody and 4,6-diamidino-2-phenylindole (DAPI)-stained to visualize all nuclei. Immunofluorescent photographs taken with FITC filters to show *lin-14* protein staining or DAPI filters to show all nuclei are shown. All views are lateral. *a*, FITC, wild-type embryo at about 7 hours of embryogenesis. Bright nuclear *lin-14* protein staining can be seen in hypodermal nuclei. *b*, FITC, wild-type larval stage one (L1) animal with the plane of focus at the midline, showing intense *lin-14* protein staining in the large intestinal nuclei. Note that there is no nucleolar *lin-14* protein staining. Below the intestinal nuclei, the smaller nuclei of the ventral cord neurons stain with the antibody. Towards the anterior the *lin-14* protein staining nuclei of the nerve ring can be seen. *c*, FITC, wild-type early L1 animal with the plane of focus at the lateral hypoderm, showing protein staining in the lateral hypodermal blast cells V1, V2, and V3, the ventral blast cells, P1/2, P3/4, and P5/6, the neurons CAN and ALM, the embryonic hypodermal nuclei Cpaapa, Cpaap and Cpaaaa, and in the neuronal nuclei of the nerve ring. *d*, FITC, wild-type mid-larval stage-one animal with the plane of focus at the midline, posterior towards the inside of the spiral. On the inner edge of the spiral, pairs of *lin-14* protein staining nuclei from ventral cord neurons can be seen. Interspersed between these *lin-14* staining nuclei are the Pn.a-derived neural nuclei and Pn.p ventral hypodermal nuclei that do not accumulate the *lin-14* protein at this stage. The larger *lin-14* protein-staining nuclei are intestinal nuclei before the L1 division. *e*, DAPI, same animal as *d*. Note that derived in *d* many Pn.a ventral-cord neuron nuclei have not yet expressed the *lin-14* protein. *f*, FITC, Wild-type larval stage two (L2) animal with the plane of focus at lateral hypoderm. No *lin-14* staining is visible. *g*, DAPI, same animal as in *f*. View showing nuclei of the hypoderm and postdeirid neurons. A comparison of *f*, *g* with *j*, *k* shows the difference in a *lin-14* gain-of-function mutant. *h*, FITC, wild-type adult animal gonad. Shown are six mature oocyte nuclei that stain with the anti-*lin-14* antibody. Note also the lack of *lin-14* protein-staining in nuclei of somatic cells. *i*, DAPI, same animal as in *h*. The same six oocyte nuclei stained with DAPI show condensed chromosomes. *j*, FITC, Gain-of-function mutant *lin-14(n355)* larval stage three (L3) animal with the plane of focus at lateral hypoderm. Shown are hypodermal nuclei that contain the *lin-14* protein. The hypodermal nuclei contain a non-staining nucleolus. *k*, FITC, *lin-14(n536)* gain-of-function mutant larval stage three (L3) animal with the plane of focus at lateral hypoderm. *lin-14* protein staining can be seen in hypodermal nuclei (large) and neuronal nuclei (small) of the postdeirid, V5.pax. *l*, DAPI, same animal as in *k*. Note that in *k* only two of the five neuronal nuclei shown in this animal have accumulated *lin-14* protein.

**METHODS.** Indirect immunofluorescence on fixed *C. elegans* specimens: strains N2, MT355=(*n355*); MT1149=(*n536*); MT1153=(*n536n540*); GR200=(*n355n726*). About 0.1–0.5 ml of these animals were fixed in 5 ml of 2% paraformaldehyde, 80 mM KCl, 20 mM NaCl, 2 mM EGTA, 0.5 mM spermidine HCl, 0.2 mM spermine, 0.5%  $\beta$ -mercaptoethanol, 15 mM PIPES pH 7.4, to which 3 ml of *n*-heptane and 1 ml of 90% methanol, 10% 0.25 M EGTA was added<sup>20</sup>. This emulsion was shaken for 20–120 min at room temperature, then frozen in dry ice-ethanol and either immediately defrosted under warm tap water or stored at  $-70^{\circ}$  for later use. After warming, the emulsion was shaken for 60–240 min at room temperature. The fixed specimens were incubated in 5%  $\beta$ -mercaptoethanol, 1% Triton-X100, 0.1 M Tris pH 7.4 for 24 h at  $37^{\circ}$  (ref. 21). The preparation was washed extensively with PBS plus 0.5% Triton-X100 (PBST), suspended in 0.1 M Tris pH 7.4, 1 mM  $\text{CaCl}_2$ , 0.1% Triton X-100 and 1,000  $\text{U ml}^{-1}$  Collagenase VII (Sigma), and incubated at  $37^{\circ}$  for 24–48 h<sup>21</sup>. The preparation was then washed extensively with PBST and pre-incubated for a few hours at room temperature with 1% bovine serum albumin (BSA) in PBST. The fixed specimens were incubated with affinity-purified anti-*lin-14* antibody, diluted 1:10 times in PBST, 1% BSA, incubated for 24 h at room temperature, and washed for 4–24 h at room temperature with PBST, 1% BSA. The sample was then incubated at room temperature for 4–24 h with FITC-coupled goat anti-rabbit antibody (Cappel) at  $10 \mu\text{g ml}^{-1}$  that had been pre-incubated at room temperature for 4–24 h with fixed *C. elegans* specimens to remove any cross-reacting antibodies. After extensive washing in PBST, 1% BSA, the specimens were mounted on a 4% low-melting point agarose pad with  $5 \mu\text{l}$  of 1  $\mu\text{M}$  DAPI and 0.5% *n*-propyl gallate in 70% glycerol-30% 0.1 M Tris pH 9.0. Fluorescent nuclei were visualized on a Zeiss universal microscope using Zeiss filter sets 487710 (FITC) or 487705 (DAPI) and a Planapo 63 objective. Kodak TriX400 black and white film or Kodak TMY 5053 film or Fuji 400 colour film 'pushed' two f-stops in development were used for photography. No immunofluorescent staining was observed in control experiments using affinity-purified sera from pre-immunized rabbits, using the anti-*lin-14* antibodies pre-incubated with excess *lacZ-lin-14* fusion protein, or using no primary anti-*lin-14* antibody before the secondary antibody staining (data not shown). Pre-incubation of the anti-*lin-14* antibodies with excess  $\beta$ -galactosidase and *E. coli* protein extracts did not affect the immunostaining (data not shown).









temporal fates. If intermediate levels of the *lin-14* protein specify particular temporal fates, then the *lin-14* temporal switch may not be binary but in fact graded.

The generation of this temporal switch must depend both on properties of the *lin-14* gene and protein and on interactions with other gene products. The *lin-14* gain-of-function mutations disrupt this regulation. These gain-of-function mutations have been shown to delete sequences from the 3' end of the *lin-14* mRNA and must remove a negative regulatory sequence that normally mediates the down-regulation of the *lin-14* protein level during development<sup>8</sup>.

Other heterochronic genes could control developmental timing of the *C. elegans* cell lineage by participating in the generation or interpretation of the *lin-14* temporal switch. For example, a recessive mutation in the gene *lin-4* causes reiterations of early larval cell lineages equivalent to those of *lin-14* gain-of-function mutations<sup>5,6</sup>, and requires a functional *lin-14* gene for this phenotype (V. Ambros, personal communication). The *lin-4* gene product may interact with the negative regulatory site that is deleted in the gain-of-function *lin-14* mutants. Other heterochronic mutations in *lin-28*, *lin-40*, and *lin-41* cause precocious expression of late larval and adult cell lineages<sup>6</sup> (V. Ambros, personal communication). By monitoring the nature of the *lin-14* protein gradient in strains bearing mutations in these other heterochronic genes, we will be able to assess whether these genes are upstream or downstream of *lin-14* in this regulatory pathway.

Thus, the level of *lin-14* protein within nuclei decreases over time like a molecular hour glass to specify developmental time to the cell lineages affected by *lin-14* mutations. These data show that the normal temporal progression of at least some of

the cell fates observed during *C. elegans* post-embryonic development is not an emergent property of the dynamics of other developmental decisions taking place, but is under explicit control of the heterochronic genes.

The *lin-14* gene is not the only developmental clock. *Lin-14* mutant animals continue the moulting cycle, even though the fates expressed at each stage are inappropriate<sup>6</sup>. In addition, the maturation of the germ line occurs at normal times in these mutants<sup>8</sup>. These processes may be regulated by other temporal control genes.

### Pattern-formation genes and evolution

Many of the developmental control genes so far identified seem to constitute components of binary or multistate switches like *lin-14*<sup>1</sup>. It is the spatial and temporal asymmetries in the patterns of developmental control gene activity during ontogeny that make cells or sets of cells different from each other. Mutations such as the *lin-14* gain-of-function and loss-of-function mutations, which change the expression pattern of such control genes, would lead to significant morphological change and could be the main cause of the variation necessary for evolutionary change. Indeed, the *C. elegans* heterochronic mutations are analogous to the heterochronic variation between species noted in phylogenetic studies<sup>6,16</sup>. This heterochronic variation could be due to mutation in one or a few heterochronic genes like *lin-14*, rather than many mutations that incidentally change developmental timing. More generally, mutations that change the spatial, temporal or cellular asymmetries in pattern formation gene activities may be the underlying cause of the manifold forms of metazoans and may be a significant force in evolutionary change. □

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## LETTERS TO NATURE

### Does supernova 1987A contain a rapidly vibrating neutron star?

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IF the recently reported<sup>1,2</sup> 0.5-ms-period pulsed optical signal from the direction of supernova 1987A originated in a young neutron star, its interpretation as a rotational period has difficulties. The surface magnetic field would have to be much lower than expected, and the high rotation rate may rule out preferred nuclear equations of state<sup>3,4</sup>. Here we point out that a remnant radial vibration of a neutron star, excited in the supernova event, may survive for several years with about the observed (gravitationally red-shifted) period. Heavy ions at the low-density stellar surface, periodically shocked by the vibration, may efficiently produce narrow pulses of optical cyclotron radiation in a surface field of  $\sim 10^{12}$  gauss. These pulses may be modulated only slightly by a

much slower stellar rotation because of the nearly isotropic emission mechanism and smearing from the strong gravitational bending of light rays<sup>5,6</sup>. We do not attempt to explain the reported<sup>1</sup> 8-hour modulation, which may be a result of timing noise (ref. 7 and J. Katz, preprint).

Present upper bounds on the luminosity of SN1987A limit the surface magnetic field of a neutron star rotating twice a millisecond to no more than  $10^9$  gauss. Unless the field rises to  $10^{12}$  gauss in  $\sim 10^3$  yr, by the emergence of a presently buried field or from magnetothermal generation<sup>8</sup>, such a low field marks this event as very different from the Crab supernova as well as from those explosions responsible for the half-dozen other pulsar/supernova remnant associations. Moreover, the inferred rotation rate, maintained without instability, may place too severe a constraint on the equation of state of nuclear matter<sup>3,4</sup>.

Neutron-star vibrations have been discussed at some length in the literature<sup>9–11</sup>. From dimensional considerations, the fundamental radial mode period  $P$  is expected to be of the order of  $(G\rho)^{-1/2} \sim 10^{-4}$  s, with  $\rho$  the mean neutron-star density. The observed period of  $5 \times 10^{-4}$  s is close to that for a typical neutron-star model<sup>10,11</sup> (corrected for the gravitational redshift) with mass  $M \approx 1 M_\odot$  and radius  $R \approx 10^6$  cm. Non-radial and higher-



order radial modes would be damped on timescales of  $\leq 1$  yr by gravitational, neutrino and electromagnetic radiation<sup>9,10,12</sup>. A major damping source for the fundamental radial mode is the URCA neutrino-emission process<sup>13</sup>, which gives a damping time of  $\sim 10^2$  yr or longer, depending on the amplitude. However, this timescale could be reduced dramatically by several effects. (1) 'Exotic' interior states with enhanced weak interactions would magnify the radial vibration damping. These include central  $\pi$ -condensates or quark matter<sup>14,15</sup>. Confirmation of our model may rule out the presence of these in the putative neutron star produced by SN1987A, unless the neutrino emission which they can generate is suppressed by superfluid energy gaps. (2) Gravitational radiation emission would increase if there were coupling to non-radial vibrations. Such a coupling will arise when the underlying spherical symmetry is broken, for example if the neutron star is rotating. For a neutron star with a uniform density, the rotation-dependent gravitational radiation damping time is<sup>16</sup>  $\sim 2 \times 10^3 P^4$  yr, where  $P$  is the rotation period in seconds. Our model would then require a slowly rotating neutron star, with  $P \geq 0.1$  s. With such a period, a  $10^{12}$ -gauss field does not cause the neutron star spindown power to exceed the current supernova luminosity. (3) Hydrodynamic eddy diffusion and electron viscosity in the core would probably damp out an initially very large amplitude of vibration that existed when the star was still very hot.

The optical radiation is expected to originate in a region no larger than a light-travel size of 150 km. For an optical pulse luminosity of  $\sim 10^{36}$  erg s<sup>-1</sup> (18th magnitude<sup>1,2</sup> at a distance of 55 kpc) originating near a neutron-star surface, the brightness temperature  $T \geq 10^{15}$  K implies either a typical radiating-particle energy  $\geq 10^2$  GeV or coherent optical emission. X-ray measurements of the supernova luminosity<sup>17</sup> require the total power radiated by the pulsar into the supernova remnant not to exceed that emitted into the optical band by more than about  $10^3$ . Incoherent curvature radiation into the optical band is so inefficient that such an emission mechanism places a generally intolerable burden on any magnetosphere acceleration model. If electron synchrotron radiation is the source of optical emission, the local magnetic field  $B$  must certainly be much less than  $10^{10}$  G. Even with a much lower field it may be difficult to accommodate such intense optical electron synchrotron radiation. For electrons of energy  $E$  to radiate at a peak photon energy  $\epsilon$  (eV), we require

$$B \sin \alpha \leq 2 \times 10^5 \left( \frac{10 \text{ MeV}}{E} \right)^2 \epsilon \text{ gauss} \quad (1)$$

where  $\alpha$  is the electron pitch angle. A synchrotron radiation spectrum (intensity  $\sim \epsilon^{1/2}$ ) could give  $(3 \text{ eV}/\epsilon)^{1/2} \geq 10^{-3}$  of its energy in the optical band if the peak photon energy  $\epsilon \leq 10^6$  eV. For  $E \geq 10^{11}$  eV and such a value of  $\epsilon$ ,

$$B \sin \alpha \leq 2 \times 10^3 \text{ gauss} \quad (2)$$

This extreme constraint on  $B$  suggests that the radiation arises

instead from cyclotron radiation from heavy stellar surface ions,  $\text{Fe}^{Z+}$  for example. These can produce optical cyclotron radiation with a typical photon energy of

$$\epsilon \approx 3 B_{12} \frac{Z}{A} \text{ eV} \quad (3)$$

where  $B_{12}$  is the magnetic field in units of  $10^{12}$  gauss and  $A$  is the atomic number of the ion. Fully stripped energetic but nonrelativistic Fe ions can give strong cyclotron optical emission in a field  $B \approx 10^{12}$  gauss. We propose that these ions may be given the required velocities ( $v \approx 10^{10}$  cm s<sup>-1</sup> for  $10^2$ -GeV  $^{26}\text{Fe}$  ions) after the radial vibration steepens into a strong shock when it reaches the very small densities and scale heights near the stellar surface<sup>18</sup>. The shock speeds up, and ion velocity behind the shock increases as density drops sharply at the surface. In the absence of detailed numerical analysis we are unable to specify the shock details. Just after the shock the kinetic energy carried by ions will initially dominate that carried by electrons. If the local post-shock density is  $\leq 10^{-3}$  g cm<sup>-3</sup>, optical synchrotron radiation from the shocked Fe ions can compete favourably with collisional loss to the much less energetic electrons around them.

Because of the short travel time for the shock passing through the surface of the neutron star, and the short ion cyclotron lifetime ( $\leq 10^{-5}$  s), a sharp pulse may be expected within each cycle. Furthermore, because the emission is concentrated around the ion gyration frequency, the vibration-shocked surface can produce a reasonably efficient conversion of internal vibration energy to optical radiation.

The total luminosity of SN1987A sets a lower limit to the neutron-star rotation period of  $\geq 20 B_{12} L_{38}^{-1}$  ms, where  $L_{38}$  is the supernova luminosity in  $10^{38}$  erg s<sup>-1</sup>. As noted above, our model requires  $P \geq 0.1$  s. As the cyclotron emission occurs in a magnetic field that varies over the surface of the star, modulation at the stellar rotation period is expected. The amplitude of this modulation may be rather small because of the isotropic energy input from the vibration, the fairly isotropic geometry of cyclotron emission, and the strong gravitational bending of the emitted light rays<sup>5,6</sup>. However, the polarization of the emitted optical light will also vary with the stellar rotation and should be detected more easily.

Future period observations should severely test the predictions of our model; specifically the period of the neutron-star vibration (unlike that from rotation) should not increase significantly with time (although the luminosity will decrease as the vibration is damped); and the rotation period of the star should be found to be  $\geq 0.1$  s unless the vibration has been excited recently. The frequency corresponding to the peak emission in the SN1987A optical pulsar spectrum could be used to estimate the magnetic field at the surface of the neutron star (see equation (3)). The detection of X-rays from the neutron star before the vibration dies out would provide another clue as to the origin of the optical light.  $\square$

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# Was the millisecond pulsar in SN1987A spun up or born spinning fast?

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**THE discovery<sup>1</sup> of an optical pulsar in SN1987A with a period of 1,968.629 Hz raises many interesting issues, chief among them being the question of how the pulsar came to acquire such a rapid rotation rate. An obvious but possibly incorrect assumption is that it was simply born that way owing to a large specific angular momentum in the iron core that collapsed. Here we argue that this millisecond pulsar, like others observed previously, has been spun up by accretion. In this case the accreted angular momentum comes from the mixed mantle and helium core of the ejecta, of which roughly  $0.1 M_{\odot}$  fell back during the first day after the explosion. This sizeable mass, and hence angular momentum, of the re-implied material is at least partly a consequence of the blue supergiant nature of the progenitor star.**

The evolution of the helium core of a massive star should, in the later stages, be decoupled from the envelope structure of the star. One expects, therefore, that the explosion of SN1987A was very similar, except in aspects related directly to the radius of its hydrogen envelope, to a typical type II supernova. There is considerable evidence to suggest that SN1987A was not particularly unusual; this evidence comes from the neutrino burst, the nucleosynthesis, the bolometric light curve and the late-time spectrum from  $\gamma$ -ray to infrared frequencies. But we have never before seen within any young supernova remnant a pulsar having such a rapid rotation rate. Could it be that this one is in a process of rapid deceleration from a (commonly occurring) initial state of high angular momentum? The limits placed on such deceleration by the discovery observation in SN1987A suggest that it is not: observations over a 7-h period (broken into half-hour intervals) revealed no more than a 0.001-Hz drift in the frequency, so that the period derivative  $\dot{P}$  is at present less than  $10^{-14}$ . The limits are in fact even more constraining, because the variations fit a sine wave with amplitude 0.0015 Hz to better than 5%, but we will adopt the less stringent number for now. Thus  $P/\dot{P}$  is considerably greater than 1,000 yr and, unless  $\dot{P}$  increases markedly in the future, the pulsar will retain its millisecond period for a long time to come. Observations of pulsars in young supernova remnants, and pulsar statistics, admittedly of objects far older than 1987A, suggest that pulsars initially have periods of 0.01–0.5 s and magnetic fields of  $\sim 10^{12}$  gauss with a small spread<sup>2–4</sup>. Such a large magnetic moment is precluded for the SN1987A pulsar because it would then be an extraordinarily bright object, much brighter than the observed brightness of roughly 100 times that of the Crab nebula pulsar in the energy band sampled<sup>5</sup>. Why, then, does the SN1987A pulsar possess such unusual properties?

We begin by noting that there is plentiful angular momentum in the cores and mantles of massive stars. The newly discovered pulsar has an angular momentum of  $\sim 10^{49}$  erg s; even without invoking differential rotation, a B0 star on the main sequence (of mass  $17 M_{\odot}$ ) with a typical rotation period of 2 days (ref. 6) and a radius of  $8 R_{\odot}$  would have angular momentum of several times  $10^{52}$  erg s. As the star evolves through the stages of nuclear burning—burning hydrogen, helium, carbon, neon, oxygen and silicon in the core and shell—convection, in particular, transports angular momentum out of the central regions of the star. The transport coefficient for this process is unknown, as is the initial distribution of angular momentum inside the star. Thus

the star might or might not end up with a collapsing core that was rotating so fast as to be near breakup.

The iron core that collapses experiences more stages of convection than any other part of the star. Each time the core contracts to ignite a new fuel, these convective episodes sweep angular momentum out, usually to a region just inside the Chandrasekhar mass. Convective shells, especially oxygen-burning, may be efficient in carrying the angular momentum out still farther. Thus a relatively slowly rotating core of  $1.5 M_{\odot}$  could plausibly collapse within a surrounding mantle endowed with considerable angular momentum, especially near its inner edge. A very approximate estimate for the angular momentum contained within the  $2.0 M_{\odot}$  of the core at central carbon ignition is  $2 \times 10^{50}$  erg s (Fig. 7 of ref. 7; see also ref. 8). Later stages of convective carbon, neon, oxygen and silicon burning would transport angular momentum but would leave a substantial residual in the  $\sim 1 M_{\odot}$  of material outside the iron core. About  $0.1 M_{\odot}$  of this material could easily contain an angular momentum of  $10^{49}$  erg s.

Moreover, SN1987A has clearly demonstrated the importance of mixing. The composition of the heavy-element core has been stirred by Rayleigh–Taylor instabilities, some in the reverse shock as the helium core runs into its own hydrogen envelope, some powered by the decay of radioactive  $^{56}\text{Ni}$  and  $^{56}\text{Co}$ , and possibly some caused by the unknown explosion mechanism itself (which may involve neutrinos creating a sizeable bubble of radiation inside the star<sup>9</sup>). In this mixing process, angular momentum as well as composition will inevitably be distributed, and in particular some material with high specific angular momentum will be mixed inwards<sup>10</sup>.

Once the explosion has occurred and the shock wave is propagating outwards, a substantial amount of matter may fall back onto the neutron star during the reverse shock into the mantle, caused by the helium core running into the massive hydrogen envelope<sup>10–13</sup>. Before the discovery of the pulsar, it was estimated that  $\sim 0.1 M_{\odot}$  of material had fallen back in SN1987A, although the errors of the calculation permit the actual value to be several times greater (or smaller). Thus sufficient angular momentum could fall back to spin up the neutron star.

The accretion is sensitive to the density of material in the vicinity of the neutron star, which decreases with time roughly as  $t^{-3}$  until the reverse shock arrives and slows the expansion. For a red supergiant, a more typical supernova precursor, it takes ten times longer for the helium core to encounter a mass equal to its own, so the reverse shock is delayed. Meanwhile the density decreases and less matter accretes. It is estimated<sup>10</sup> that 100 times less matter would accrete in the explosion of a red supergiant than in SN1987A. This could explain why neutron stars born in more typical type II supernovae end up rotating more slowly. If so, the rapidly spinning pulsar may be yet another special attribute of the relatively small radius of the anomalously blue supergiant progenitor.

The limit to the degree of spin-up, however, lies not just in the supply of angular momentum but in the efficiency with which it can be added. We assume that material can accrete with a tangential velocity no greater than the Keplerian orbit speed. General relativity modifies the newtonian value<sup>10</sup> because there are no stable orbits inside 3 Schwarzschild radii,  $R_s$  (for a rapidly rotating neutron star this value is reduced to 1.75–2.2  $R_s$  (ref. 14)). The angular momentum delivered to a (non-rotating) neutron star of mass  $1.5 M_{\odot}$  is  $4.6 \times 10^{48}$  erg s per  $0.1 M_{\odot}$  accreted ( $3^{1/2} R_s c$  per g; (ref. 14)). This means that roughly  $0.2 M_{\odot}$  needs to have fallen back onto the neutron star. If the neutron star were still rotating highly differentially, with the crust moving much faster than the superfluid central core (which contains most of the moment of inertia), the required mass could be much less. Recent studies<sup>25</sup> suggest, however, that the crust and core are efficiently coupled and that the timescale for communicating angular momentum is quite short, certainly less

than a year. If the timescale were one year,  $\dot{P}$  would be much greater than the limit given above. For now we adopt the more restrictive assumption of rigid rotation.

The (baryon) mass of the neutron star inferred from the neutrino burst is in the range  $1.2\text{--}1.7 M_{\odot}$  (ref. 16) with most probable values near the middle of the range; clearly, an addition of  $0.2 M_{\odot}$  to a  $1.5 M_{\odot}$  neutron star would give a mass of  $1.7 M_{\odot}$ . The maximum mass of a hypothetical neutron star<sup>17,18</sup> having equation of state 'F' (which is relatively 'soft') and a period of 0.5 ms is  $1.87 M_{\odot}$  (for which the moment of inertia is  $1.16 \times 10^{49}$  erg s). Without rotation, a neutron star of mass  $1.7 M_{\odot}$  would be unstable to collapse for such an equation of state. Although the gravitational masses (roughly 10% less than the baryon mass) of neutron stars in our Galaxy may be less than  $1.5 M_{\odot}$  (ref. 19), the new pulsar probably needs to be more massive than this in order to rotate as rapidly as it does<sup>17,18</sup>. Accretion, as described here, would produce the required mass increase naturally.

Most of this mass addition would have occurred early on, with a maximum rate occurring soon after the reverse shock reached the core (that is, 2 hours after the explosion<sup>20</sup>). Most of the accretion energy would have been expelled in the form of neutrinos<sup>21</sup>. And additional energy radiated would be negligible compared to the supernova energy on day 1. Although our model has a large accretion rate in the early phases, this can be reduced by the escape of radiation from the vicinity of the neutron star after about 7 months (ref. 10). The present accretion rate onto the pulsar must be very small so as to accommodate the known bolometric luminosity of  $3 \times 10^{38}$  erg s (R. Catchpole, personal communication). All radiation with energy below 10 keV would still be effectively converted to light in the optical and infrared bands<sup>22</sup>, so the present accretion rate is below about  $10^{-8} M_{\odot} \text{ yr}^{-1}$ . For such a small  $\dot{M}$  (and  $\dot{P}$ ), the pulsar is in no immediate danger of becoming a black hole. If added at the Keplerian velocity, accretion at a rate of  $10^{-8} M_{\odot} \text{ yr}^{-1}$  would contribute angular momentum to the neutron star at a rate several orders of magnitude below the limit inferred from  $\dot{P}$  in the observations.

The mass addition could also have the interesting effect of reducing the surface magnetic field strength, as a result of an initially very high accretion rate of material with a disordered magnetic moment, which could bury the magnetic field of the accreting neutron star. A field of  $10^{12}$  gauss would be overwhelmed by an accretion rate of only  $0.002 M_{\odot}$  per day, which is considerably less than the rate expected. Alternatively, convection and nuclear activity in the accreted material may have reduced the surface field<sup>23</sup>. An interesting consequence of the model proposed above is that the magnetic dipole field of the core, initially buried by accretion, may eventually emerge, leading to a brightening of the pulsar and a more rapid spin-down.

There are surprisingly few major observational differences between a pulsar born rotating fast and one spun up during the first day of its life. The latter would certainly have a greater mass, but not so great as to be incompatible with the proposed nuclear equation of state. To distinguish between the two may require a consideration of all observations and models of the supernova, paying special attention to the explosion mechanism. Our model offers the advantage of explaining why this pulsar is unusual and of coupling this peculiarity to the structure of the progenitor star Sk-69 202. Given that a plausible explanation for the blue nature of that star is, at least in part, a manifestation of the low metallicity of the Large Magellanic Cloud<sup>20,24</sup>, one implication of the model is that similar millisecond pulsars may have been created in other metal-deficient regions. However, there are other factors besides metallicity that are involved in the evolution of a blue supergiant progenitor<sup>24</sup>. These include the mass of the star ( $15 \leq M/M_{\odot} \leq 22$ ), the efficiency of semi-convection (which is small), and the amount of mass loss (also small). It is unlikely that all pulsars in the LMC would be born

as millisecond pulsars, but it is possible that some could. Pulsar 0540-69, for example, which is also in the LMC and within a remnant of estimated age 800 yr (ref. 25), has a 'normal' period of 50 ms and probably also a strong magnetic field.

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## Geminga and the search for optical counterparts of $\gamma$ -ray-burst sources

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THE nature of gamma-ray-burst (GRB) sources has remained a mystery, in part because of the lack of any optical identification. Recently, deep CCD photometry has identified the optical counterpart G' to the  $\gamma$ -ray source Geminga, whose location on a colour-magnitude diagram is unique<sup>1</sup>. Although the X-rays from G' are probably due to thermal emission from the neutron star, the implied column density ( $\geq 5 \times 10^{20}$ ) is inconsistent with the distance ( $D < 100$  pc) required to fit the optical flux to the same spectral component. Here I show that the optical emission could be due instead to a cold accretion disk with accretion rate  $\dot{M} \approx 10^{11} \text{ g s}^{-1}$ . This has promising implications for the search for the optical counterparts to GRB sources whose basic physical parameters may resemble those of radio pulsars and related objects such as Geminga. I argue that a similar search in the field of a GRB location should produce candidates similar to G' if  $20 \text{ pc} \leq D_{\text{GRB}} \leq 500 \text{ pc}$ , as predicted by the disk-reprocessing model for the associated optical transients. This possibility is discussed in the light of the fact that the first optical counterpart to a GRB source may already have been found, which, if it is confirmed, may be used to test the accretion-disk model.

The confirmed optical identification of 1E0630+178 with a 25-mag blue object (G'') makes it very likely that this X-ray source is an isolated neutron star and strongly suggests that it is the counterpart to the high-energy  $\gamma$ -ray source Geminga<sup>1</sup>. The detection of thermal emission from a neutron star is not new. The discovery of G' is significant, however, because of its



similarity to Vela-type pulsars and because of the possibilities implied in respect of the search for the quiescent counterparts of GRB sources, which may themselves be isolated, magnetized neutron stars.

Although the basic mechanism for producing GRBs is not yet well understood, support for the neutron-star model is extensive<sup>2</sup> and has been augmented by the confirmation of low-energy features (interpreted as cyclotron resonances) in some GRB spectra<sup>3-8</sup>. It is not surprising, therefore, that GRB sources have sometimes been associated with extinct pulsars<sup>9-11</sup>. The similarities between the two include the following. (1) Both seem to be isolated neutron stars<sup>12-14</sup>. (2) At least some of the GRB sources, like pulsars, are highly magnetized (with surface magnetic fields  $\geq 10^{12}$  gauss (refs 5, 6)). (3) Several pulsars (including PSR0531+25 and 0833-45) emit detectable fluxes of  $\gamma$ -rays up to energies in excess of  $2 \times 10^3$  MeV (ref. 15), with a flux density that falls off as a power law characterized by an index  $\alpha \approx -1$ . Significantly, the Gamma-Ray Spectrometer on the Solar Maximum Mission satellite has discovered that emission of  $>1$  MeV (with  $-2 \leq \alpha \leq 0$ ) is a common component of GRBs, and that in many cases it actually dominates the emitted power<sup>6,16</sup>. (We note, however, that GRB spectra below  $\sim 1$  MeV are not always similar to those of rotation-powered pulsars.) (4) There is some evidence that GRB sources may have periods<sup>17</sup> similar to those of pulsars<sup>18</sup>. However, the pulsar period distribution peaks around  $\sim 0.5$  s (and extends down to 1.6 ms in the case of PSR1937+214), whereas the periods in GRBs (if confirmed) fall in the range  $\sim 1$ -10 s. Although this distinction may be simply a selection effect (that is, faint, slow pulsars are undetectable at large distances, and GRB periods  $\ll 1$  s may be 'hidden' by the intrinsic rapid variability of the burst flux), it might also be an indication that GRB sources are indeed extinct pulsars and that the transition from one population to the next occurs when the spin period of the neutron star exceeds  $\sim 2$  s (ref. 11). On the other hand, it might simply indicate that although the two types of systems have similar properties, they form by different processes which lead naturally to different period distributions<sup>19</sup>.

Is it possible then, in view of this close analogy between Geminga-type sources and GRB sources, to predict the observable characteristics of the optical counterparts to the latter? Before addressing this question, let us consider the status of

Geminga and its identification with G'. Deep CCD photometry<sup>1,20</sup> at the Einstein Observatory shows that the star in question is by far the bluest object in the field of the Einstein error circle of the X-ray source 1E0630+178 (Fig. 1). Even though deep optical searches reaching beyond 25 mag reveal many objects, a source such as G', with a large and significant departure from colours expected and observed from the field stars of similar magnitude, occupies a distinct, outlying position on a colour-magnitude diagram. Quantitative photometry and reanalysis of the Einstein data led Halpern and Tytler<sup>1</sup> to conclude that the optical and X-ray emission together are consistent with a single-temperature black-body spectrum of temperature  $T = (4.3 \pm 1.7) \times 10^5$  K, although the X-ray data alone are best fitted by  $T = 1.1 \times 10^6$  K. This is an important distinction because the lower temperature implies a distance  $D < 100$  pc, whereas the higher temperature requires both that  $D < 2$  kpc and that the optical emission must have another source. In view of the fact that the column density  $N_H$  corresponding to the single-temperature fit is at least  $5 \times 10^{20}$  (which is much larger than the measured values within 100 pc of the Sun in this direction), a line of sight with this value of  $N_H$  would more probably extend to  $\sim 500$  pc. There is thus an inherent difficulty with the single-temperature model. (One should keep in mind, however, that the surface composition of isolated neutron stars is poorly known and a black-body fit to the X-ray spectrum may not be adequate<sup>21</sup>.) A second objection to the smaller distance implied by the single-temperature fit is the correspondingly small displacement of the source away from our galactic plane ( $\leq 7$  pc), which is statistically unlikely<sup>1</sup>.

The optical emission from G' may be due to a distinct (perhaps synchrotron<sup>1</sup>) component. I propose that at least part of this optical flux may be emitted by a cold accretion disk surrounding the neutron star. Systems such as this have already been considered in disk models for pulsar action<sup>22</sup>, and as possible  $\gamma$ -ray-burst sources<sup>10,23,24</sup>. In addition, there is some evidence that these disks are necessary to account for the optical transients associated with some GRBs<sup>14,25,26</sup>.

To explore this possibility, I have assumed a model in which the optical and X-ray fluxes are due to a thermal, isotropically radiating neutron star<sup>27</sup> with a temperature  $T_0 = 1.1 \times 10^6$  K, mass  $M = 1.4 M_\odot$  and radius  $R = 10$  km, and a cold disk whose emission may be treated as a sum of black bodies<sup>6,14</sup>. The maximum disk temperature,  $T_{\max} = [3GM/65\pi\sigma R_{\max}^3]^{1/4} = 1.22 \times 10^5 (M/1.4 M_\odot)^{1/4} (R/10 \text{ km})^{-3/4} \eta^{-3/4}$  K, is attained at the equatorial radius  $R_{\max} = (49/36)R_{\text{in}}$ , where  $R_{\text{in}} = \eta R$  defines the inner edge of the disk and  $\eta$  ( $\sim 3$  for the systems considered here<sup>6,14</sup>) is a parameter characterizing the Alfvén radius. Thus, accretion disks driven by ordinary degenerate electron viscosity have temperatures (corresponding to accretion rates  $\dot{M} \approx 10^{10} \text{ g s}^{-1}$ ) that are too low for them to be detected from X-ray emission, but that permit them to contribute significantly to the optical flux because of their large emitting surface area.

Figure 1 shows the magnitude and colour expected of G', together with the published data<sup>1</sup>. Because  $\eta \approx 3$ , only the parameter  $\dot{M}$  can influence the result significantly. (The inclination angle  $i$  of the disk to the line of sight enters only through a factor of  $\cos i$ , which I will assume to be 0.7 in all the calculations.) For a fixed  $\dot{M}$ , the range in the  $g$  magnitude reflects the uncertainty in the source distance  $D$ , whereas the extent in  $g-r$  on the colour-magnitude diagram corresponds to the unknown degree of reddening  $A_g$ . (The value  $A_g = 1.7$  is the maximum observed in this direction<sup>1</sup>.) Once the neutron-star temperature and distance ( $\sim 500$  pc) are fixed by the X-ray data, the optical data, which provide a colour and magnitude, can be fitted with just one essential parameter ( $\dot{M}$ ). The diamond-shaped region shown in Fig. 1 corresponds to an accretion rate  $\dot{M} = 10^{11} \text{ g s}^{-1}$ . Note that, for these parameter values, a distance  $D \approx 500$  pc would imply an amount of reddening corresponding to  $A_g \sim 0.3$ -0.4.

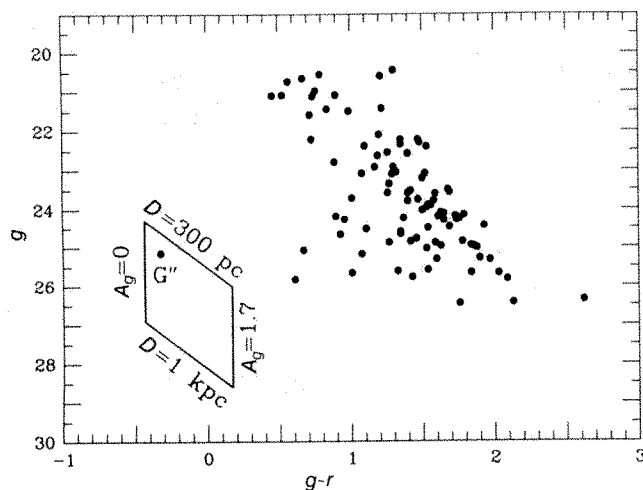


FIG. 1 Colour-magnitude diagram for the stars in the field of the Einstein error circle for the X-ray source 1E0630+178 (Geminga). At a distance  $300 \text{ pc} \leq D \leq 1 \text{ kpc}$ , thermal emission from the surface of a neutron star with a temperature ( $\sim 1.1 \times 10^6$  K) fitted to the X-ray data cannot account for the optical flux. The region enclosed by the diamond corresponds to the magnitude and colour of an accretion disk with accretion rate  $\dot{M} \approx 10^{11} \text{ g s}^{-1}$ .  $A_g$  is the reddening in the direction of this source. (Data are taken from ref. 1.)

The success of this fit is promising in so far as GRB sources are concerned because they may contain cold accretion disks<sup>10,14,23-26</sup>. Application of the disk-reprocessing model to the optical transients associated with GRBs<sup>14,19</sup> predicts distances in the range  $\sim 20$ –500 pc for the four known optical-transient (OT) GRB sources, not unlike that of Geminga. Of these, the OT1928/GRB1978 object<sup>28</sup> is predicted by this model to be the closest, with  $20 \text{ pc} \leq D \leq 150 \text{ pc}$ . Perhaps not coincidentally, this also happens to be the GRB source for which the best attempts have been made to search for a quiescent optical counterpart<sup>29,30</sup>.

The colour-magnitude data for all those sources in the field of the OT1928 error box for which colour information is available<sup>30</sup> are shown in Fig. 2. Although these sources are much fewer in number than those in Fig. 1, the similarity of the two plots is nonetheless apparent. Also shown in this figure is the range in  $B$  and  $B-V$  predicted on the basis of the simple neutron-star-plus-disk model described above. In this case, the amount of reddening is expected to be very small ( $A_B \approx 0$ ) because the source is relatively close. The allowed range in  $B$  and  $B-V$  is instead due to the uncertainty in the accretion rate and distance. As before, standard parameter values were chosen for the neutron star, which was also assumed to have a surface temperature  $T_0 \approx 10^5 \text{ K}$ , consistent with the most constraining X-ray observations currently available for this source<sup>31</sup>.

As was the case for  $G''$ , there is no question that the source DD is unique among the objects detected in the field of the OT1928 error box: main-sequence stars with  $B \geq 25$  and  $B-V \leq 0$  would have to be early types with  $D \approx 10 \text{ kpc}$ , which is unlikely given that the galactic latitude of this field is  $\sim -84^\circ$ , and active galactic nuclei with this colour are typically much brighter ( $V < 20$ ). CC has a magnitude similar to DD, but its colour and magnitude together are consistent with a normal late-K-type main-sequence star. The proximity of DD to the predicted range of  $B$  and  $B-V$  suggests that, if this object is indeed an isolated neutron star surrounded by an accretion disk, then  $10^7 \text{ g s}^{-1} \leq \dot{M} \leq 10^9 \text{ g s}^{-1}$  and  $D \approx 100 \text{ pc}$ , as predicted by the disk-reprocessing model<sup>14</sup>. Thus, in conjunction with deep CCD photometry to identify more precisely the magnitude and colour of DD, confirmation of candidate DD as the burst source would strongly support this model, whereas ruling it out would argue against it. If this model is correct and the connection between GRB sources and pulsars is borne out by further observational

testing, the longer periods (if confirmed), the lower neutron-star surface temperatures and the apparently lower accretion rates in GRB sources argue either for an older (perhaps extinct) branch of the pulsar population or for two distinct populations differing only in the scale of the physical parameters characterizing their constituents.

Obtaining CCD photometry to a limiting magnitude ( $5\sigma$  detection threshold) of 26 in  $B$ ,  $V$  and  $R$  band-passes is feasible in principle at prime focus on a 4-m telescope. Although the historical OTs (found on archival photographs) and the modern-day GRBs are separated by as much as 40 years, the sources will not have shifted position by more than  $\sim 9''$  for a transverse velocity of  $\leq 50 \text{ km s}^{-1}$  at a distance of 200 pc. Because of its unique colour, any GRB source within the CCD's  $\sim 4' \times 4'$  field of view centred on the OT location should stand out clearly in a colour-magnitude diagram. Such an effort should at least be carried out for the source DD, which is arguably our strongest candidate for an optical counterpart to a GRB source.  $\square$

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## Evidence from Voyager II photometry for early resurfacing of Umbriel

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THE uranian satellite Umbriel's dark, heavily cratered surface is remarkable for its apparent uniformity in Voyager II images<sup>1</sup>. Its most conspicuous geological feature is a comparatively high-albedo, annulus-shaped deposit which covers the floor of the 40-km diameter crater Wunda<sup>1</sup>. Here we present new Voyager II albedo maps of Umbriel which reveal that its surface is subdivided into low-contrast, crudely polygonal areas ranging in size from tens to hundreds of kilometres (Fig. 1). The largest polygons are elongate with systematically trending northeast-southwest boundaries. Some of the polygonal areas form topographic depressions several

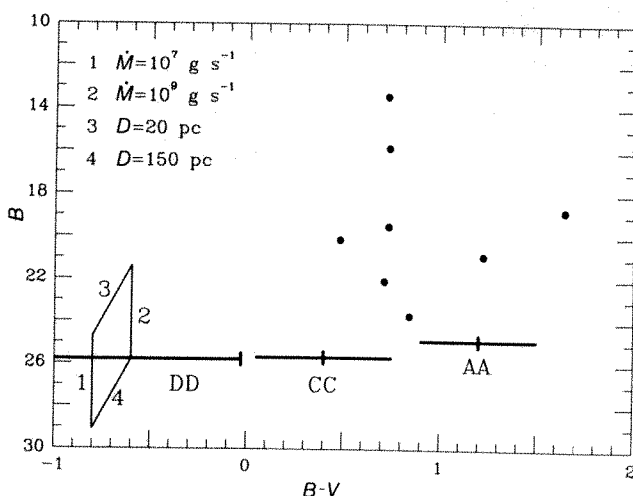


FIG. 2 Colour-magnitude diagram for the stars in the field of the 1928 optical transient, which seems to be associated with the 1978 November 19  $\gamma$ -ray burst. As is the case for  $G''$ , the position of source DD is unique. Its proximity to the region of  $B$  versus  $B-V$  predicted by the 'single-neutron-star plus cold-disk' model makes it a very strong candidate for the optical counterpart to this GRB source. (Data are taken from ref. 30.)



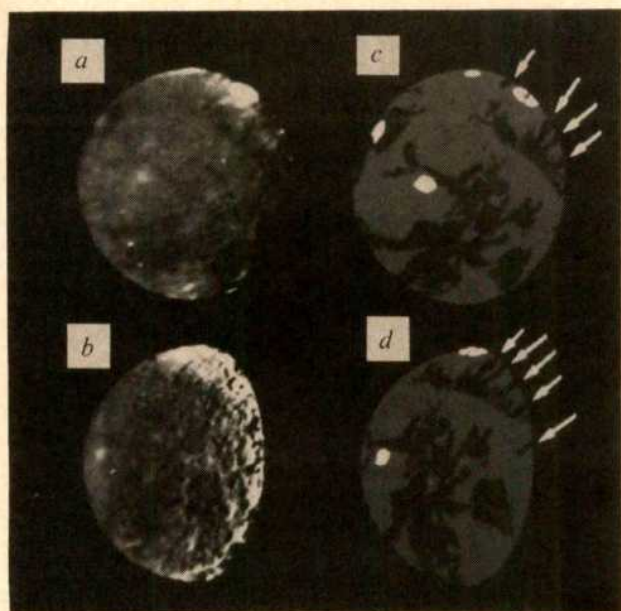


FIG. 1 *a*, Voyager image FDS 26825.51 after shading correction and contrast enhancement. Phase angle is  $31^\circ$  and resolution is 16 km per line pair. *b*, Highest-resolution Voyager image (FDS 26840.04), processed as in *a*. Phase angle is  $56^\circ$  and resolution is 8 km per line pair. *c*, *d*, Sketch maps showing locations of features identified in our albedo map (Fig. 2*a*) as they would appear in images at left. Arrows identify the locations of parallel topographic grooves. The brightest features are bright craters. Irregularly shaped dark areas are prominent dark polygonal terrains.

kilometres in depth. We suggest that this newly discovered global albedo pattern is a relic of the early tectonic disruption of Umbriel's surface.

We have prepared a new, photometrically accurate map of Umbriel's surface which reveals a low-contrast global albedo pattern not recognized previously (Fig. 2*a*). This map is a composite constructed by first correcting the brightnesses in each Voyager image to compensate for differences in viewing and illumination geometry (Fig. 1*a*, *b*), and then projecting the image into standard polar stereographic coordinates. The digital map in Fig. 2*a* represents a mosaic of four such projected images (Voyager FDS 26788.15, 26797.22, 26825.51, 26840.04).

To perform shading corrections to each image, we applied the analytical equation of Hapke<sup>2-4</sup>, which describes the variation of surface brightness of rough particulate surfaces with changing photometric geometry. For any given surface element, photometric geometry is specified by three angles: incidence angle,  $i$ , emission angle,  $\epsilon$ , and phase angle,  $\alpha$  (the angle subtended between two vectors pointing from the surface to the Sun and to the observer). Hapke's equation requires five model parameters whose values we determined from observations of Umbriel's changing brightness during different phases of the Voyager II encounter. Two of the parameters,  $\bar{\omega}_0$ , the average particle single-scattering albedo, and  $g$ , the asymmetry factor in the Henyey-Greenstein<sup>5</sup> particle-phase function, are closely related to the composition and mechanical structure of soil particles. The opposition effect (a surge in brightness observed in particulate surfaces at low illumination and small viewing angles) is described by two parameters:  $h$ , which relates the angular width of the surge to the state of compaction of surface particles, and  $B_0$ , which describes the amplitude of the opposition surge. Macroscopic surface roughness is described by Hapke's  $\bar{\Theta}$  parameter, the average slope angle of sub-resolution-scale topographic relief.

Preliminary global-average values of these parameters have been determined from 'clear-filter' ( $\sim 0.48 \mu\text{m}$ ) observations by Voyager of Umbriel's integrated-disk brightness<sup>5</sup>. That study

provides the best available estimates of opposition surge parameters ( $B_0 = 2.01$ ,  $h = 0.06$ ), but improved estimates of  $\bar{\omega}_0$ ,  $g$  and especially  $\bar{\Theta}$  can be obtained on the basis of Umbriel's disk-resolved photometric behaviour. Accordingly, we have collected disk-resolved measurements of Umbriel's brightness from five clear-filter Voyager images (FDS 26788.15, 26797.22, 26825.51, 26840.04, 26904.04) covering phase angles from  $10.4^\circ$  to  $142.9^\circ$  and averaged in bins of  $5^\circ$  of photometric latitude and longitude over each visible disk (excluding the few visible bright craters). Optimal values of  $\bar{\omega}_0 = 0.35$ ,  $g = -0.18$  and  $\bar{\Theta} = 26^\circ$  were then determined by fitting Hapke's equation to these data using a nonlinear least-squares algorithm<sup>6</sup>.

Figure 1*a* and *b* shows the highest-resolution images of Umbriel after performing the shading correction and after contrast stretching to enhance details. Quasi-polygonal discontinuities between coherent regions of lower and higher albedo can be identified clearly in Fig. 1*a* and, less easily, in Fig. 1*b* (for which resolved topographic texture more strongly obscures albedo markings). The fact that these features become more distinct with decreasing phase angle suggests that they are indeed albedo markings rather than shadows cast by topography. Some of the discontinuities define closed, approximately polygonal areas (Fig. 1*c*, *d*), ranging from less than 50 km to greater than 600 km in size. In many cases, albedo contrast between adjacent polygonal areas is too variable along the boundaries to allow clear definition of complete polygons.

In Table 1 we list normal albedos (the reflectance at normal incidence and emission angles) of selected features on Umbriel. The average normal albedo of Umbriel is 0.21. The brightest feature on the satellite, the bright annulus crater, has an albedo of 0.49 and a contrast of 40%. In comparison, the apparent albedo contrast of probable bright-crater ejecta is only about 5%. The photometric contrast between the darkest polygons and typical surrounding regions is only 2.4%.

The composite albedo map can be used to examine the distribution of dark polygons over Umbriel's visible surface. On scales of less than about 100 km, polygons seem to have random orientations. However, sub-global-scale polygons at  $25^\circ$  S,  $300^\circ$  E and  $73^\circ$  S,  $270^\circ$  E are elongated, with nearly parallel northeast-southwest-trending lengthwise boundaries. A pattern of parallel topographic grooves (shown by arrows in Fig. 1*c* and *d*) cross-cuts the long boundary of one of these polygons at large angles.

At the highest resolution possible for Voyager (11 km per line pair), it is difficult to identify visually any possible topographic boundaries that may be separating polygons. Thomas<sup>7</sup>, however, has recently derived accurate elevation profiles along the limb of Voyager image FDS 26840.04, which projects into our albedo map along the arrow labelled A-A' in Fig. 2*a*. In Fig. 2*b* and *c*, we compare the limb elevation profile of Thomas with the corresponding normal albedos along the length of the limb projection in Fig. 2*a*. A close, but not perfect, correspondence between albedo and topography is evident: areas of lower albedo are topographically lower, and higher albedos are associated with topographic highs. We stress that the topography shown

TABLE 1 Clear-filter normal albedos for selected features on Umbriel

Feature	Location	Normal albedo
Global average		$0.21 \pm 0.02$
Darker polygons	$19^\circ$ S $310^\circ$ E	$0.202 \pm 0.011$
	$65^\circ$ S $7^\circ$ E	$0.191 \pm 0.004$
Brighter polygons	$83^\circ$ S $135^\circ$ E	$0.204 \pm 0.002$
	$83^\circ$ S $98^\circ$ E	$0.209 \pm 0.002$
	$56^\circ$ S $257^\circ$ E	$0.202 \pm 0.002$
Bright craters	$82^\circ$ S $270^\circ$ E	$0.232 \pm 0.007$
	$42^\circ$ S $192^\circ$ E	$0.226 \pm 0.004$
Bright annulus	$5^\circ$ S $273^\circ$ E	$0.491 \pm 0.051$



was determined from limb geometry, that is, by a method totally independent of photometry (which is to say, of albedo).

There is no obvious stratigraphic relationship between darker and brighter polygons; but their existence, along with systematic patterns of global orientation and the nature of the topography, is consistent with the interpretation that they are relics of tectonic breakup and resurfacing of Umbriel. The tendency for lowest-albedo materials to occur in low-lying terrains is consistent with an internally driven resurfacing event involving darker materials. Such darker materials are known to have been extruded on other uranian satellites, that is, certainly on Miranda and probably on Oberon.

The apparent muting of topographic boundaries between polygons by accumulated impact craters suggests that the proposed resurfacing occurred very early in Umbriel's geological history. Indeed, Strom<sup>8</sup> has suggested, on the basis of cratering statistics, that Umbriel was resurfaced earlier than other uranian satellites. However, any geological interpretation of the global albedo pattern discovered in our study must be seen in the light

of the very low spatial resolution of the Voyager images of Umbriel. A global quasi-polygonal, low-contrast albedo pattern definitely exists on Umbriel. Limited topographic data suggest that a correlation between albedo and topography exists. The geological interpretation of these patterns is uncertain. □

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## Influence of long-range transport of combustion emissions on the chemical variability of the background atmosphere

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MAUNA Loa Observatory, located 3,400 m above sea level on the island of Hawaii in the middle of the Pacific Ocean, is a critical site for determining the background chemical reactivity of the unpolluted atmosphere and for monitoring its rate of change on a global scale. However, recent measurements of soluble nitrogen (principally  $\text{HNO}_3$ ) at the observatory find mixing ratios rising from their expected background values of 0.02–0.03 parts per  $10^9$  by volume (p.p.b.v.) in the winter to 0.07–0.12 p.p.b.v. in late summer with three-hour events as high as 0.25 p.p.b.v. (ref. 1). This raises the specific question of contamination by the long-range transport of pollution<sup>1</sup> and a broader question of the chemical variability of the background atmosphere. Here we show that a general circulation transport model which simulates the global spread and deposition of emissions from fossil-fuel combustion can reproduce the nitrogen measurements at the Mauna Loa Observatory. By isolating individual source regions, we show that US emissions are responsible for the late summer increase and that Asian emissions cause a smaller increase in the spring. These simulations, together with the earlier observations, indicate frequent contamination of the Mauna Loa Observatory by the long-range transport of reactive trace gases, such as  $\text{HNO}_3$ , and suggest a highly variable background atmosphere. It is essential that we are aware of such variability in order to discern anthropogenic effects on the atmosphere.

We use a global transport model which has already demonstrated the important role of dry deposition in acid deposition over North America<sup>2</sup> and the impact of combustion nitrogen on the global nitrogen budget<sup>3</sup>. The model has 11 altitude levels (31.4, 22.3, 18.8, 15.5, 12.0, 8.7, 5.5, 3.1, 1.5, 0.5 and 0.08 km), a horizontal grid size of ~265 km, and a time step of ~26 min (ref. 4). Using the 6-h time-average winds and precipitation provided by a parent general circulation model<sup>5,6</sup>, the collection of gaseous and particulate reactive nitrogen compounds that result from the combustion emissions are transported as a single tracer,  $\text{NO}_y$ . We have already discussed the formulation of tracer transport<sup>3,4</sup>, the global source of nitrogen resulting from fossil-fuel combustion<sup>3</sup>, the effective dry deposition of  $\text{NO}_y$ <sup>3,7</sup> and the effective removal of  $\text{NO}_y$  by precipitation<sup>3,4</sup>.

Those emissions not deposited in the source region become

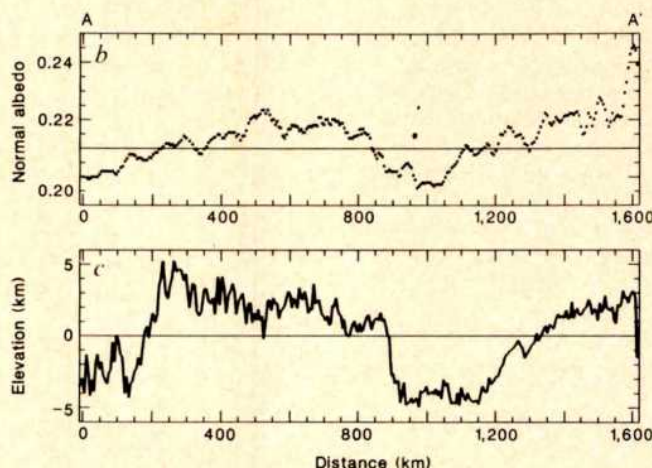
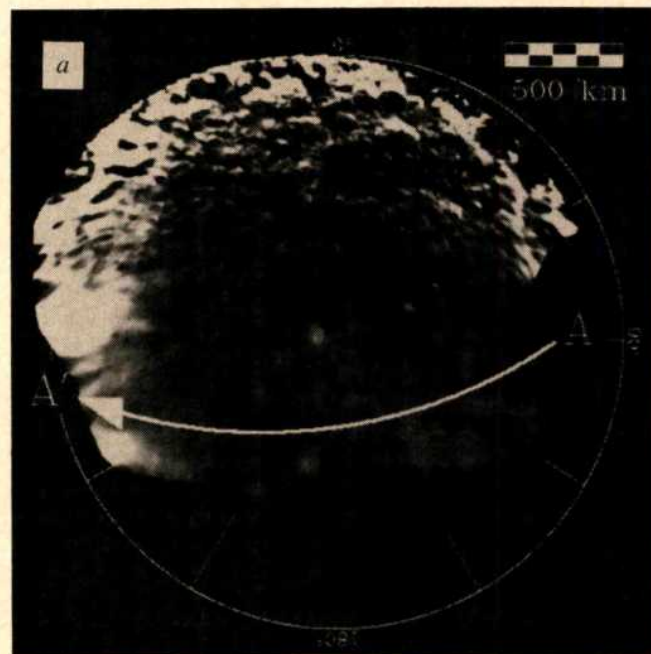


FIG. 2 *a*, Composite polar stereographic projection of four Voyager images of Umbriel. Arrow A–A' identifies the projected location of the limb from image FDS 26840.04 (Fig. 1*b*). *b*, Plot of normal albedos from *a*, along section A–A'. *c*, Elevation profile along section A–A', corresponding to albedo plot in *b*.



available for long-range transport. This available  $\text{NO}_x$  is specified by basing the model's dry deposition and precipitation removal on measurements of nitrogen deposition in precipitation and of surface concentrations of individual nitrogen species. A single linear parameter is adjusted to bring the model's yearly integral of precipitation removal over North America into agreement with observation<sup>2</sup>. This same parameter leads to a good simulation of the yearly wet deposition of nitrogen over the European source region and remote locations in the Northern Hemisphere<sup>3</sup>. Dry deposition uses effective deposition velocities for  $\text{NO}_x$  which are based on the surface concentrations of the individual reactive nitrogen species measured over the United States.

The collective transport and parameterized deposition of  $\text{NO}_x$  precludes the explicit transport of individual nitrogen compounds, particularly insoluble species, such as peroxyacetyl nitrate (PAN) and other organic nitrates. However, we find that much of the transport from Asia and North America to the central Pacific is in stable subsiding air with little precipitation, and that the simulated  $\text{NO}_x$  deposition and surface concentrations in the central Pacific agree reasonably with observations<sup>3</sup>. The controlling factor is the model's meteorology—which both lifts the  $\text{NO}_x$  into the free troposphere (that region of the troposphere that is not directly influenced by boundary-layer mixing) and transports it to Hawaii—and not the detailed chemistry in the source region and along the transport path.

In Fig. 1, we compare the measurements of soluble nitrogen at the Mauna Loa Observatory<sup>1</sup> with the simulated monthly average mixing ratios of  $\text{NO}_x$  in grid boxes located over Hawaii. The model's simulation of  $\text{NO}_x$  at 940-mbar level in the atmosphere,  $\sim 0.5$  km above sea level, reproduces both the magnitude and seasonal variation of the observations from the Mauna Loa Observatory. In contrast, the simulated mixing ratios at the 685-mbar level, approximately the height of the Mauna Loa Observatory, agree only with the low winter levels and show no increase in the other seasons. The model does not have sufficient horizontal and vertical resolution to simulate the island of Hawaii, let alone the two mountain peaks and the local complex flow. It simulates long-range transport to grid boxes in the vicinity of Hawaii, and the 685-mbar grid box represents the free troposphere over the Pacific rather than the Mauna Loa Observatory.

Furthermore, extremely high-resolution ( $10 \text{ km} \times 10 \text{ km}$ ) simulations of the air flow around Hawaii show that the normal winds at 3,400 m in the free troposphere are greatly modified by the mountainous terrain<sup>9</sup>. Air parcels are carried up from below in direct flow from the windward side of the mountain and also in return flow from eddies that form in the lee of the island. As a result, the air sampled at the Mauna Loa observatory is a complex mixture of normal free-tropospheric air found at 3,400 m, air from stable layers below 3,400 m and above the maritime boundary layer, air from the maritime boundary layer (that is, below the maritime inversion layer and above the sea surface) and air from the island's boundary layer, which is likely to be contaminated by local pollution and natural emissions. Coincident measurements of humidity, wind direction and condensation-nuclei concentration are used to delete soluble nitrogen observations that may be contaminated by local pollution and natural emissions from both the island and the sea<sup>1</sup>, but measurements in air from stable layers below 3,400 m are retained. Over the ocean, the model produces a stable layer above a weakly mixed maritime boundary layer that is  $\sim 1.5$  km thick with a well mixed lowest level of  $\sim 0.2$  km. This structure is in qualitative agreement with the observed maritime boundary layer over the eastern tropical Pacific<sup>8</sup>, although the observed thickness increases from  $\sim 0.5$  km near the Baja to 1.5–2.0 km near Hawaii.

By running separate integrations for the global, Asian and US sources, we are able to identify the individual contributions. The observed summer mixing-ratio maximum, which is well

simulated in the model at 940 mbar, results from a sharp increase in transport from the United States to Hawaii that continues through the autumn. During the winter and spring, nitrogen emissions from Asia dominate and produce the spring increase. The low background levels at the 685-mbar level, with a hint of a spring maximum, are completely dominated by emissions from Asia, with little contribution from the United States or the rest of the globe.

A more complete picture of the atmospheric transport is provided by the simulated time series of  $\text{NO}_x$  at the 940-mbar level over Hawaii in Fig. 2. We see a collection of discrete transport events superimposed, at a frequency of 3–5 days, over a very low background. In particular, we see that the summer maximum is the result of a few large events. The limited observations appear to have a similar character (B. J. Huebert, personal communication). Both this simulation and the observations at Mauna Loa Observatory suggest that the levels of reactive gases in the remote atmosphere may be highly variable.

During the summer, in both the model and the real atmosphere, the time-mean Pacific subtropical high moves from a latitude of  $\sim 20^\circ \text{N}$  off the Baja to a position north of Hawaii at  $\sim 35^\circ \text{N}$  (ref. 10). At times, this produces a subsiding flow in the lower troposphere from the south-west United States and Mexico to Hawaii. When combined with surface transport from the source regions and small-scale vertical mixing over arid land, episodic transport of  $\text{NO}_x$  to the vicinity of Hawaii occurs in the model. Three-dimensional trajectories and a detailed

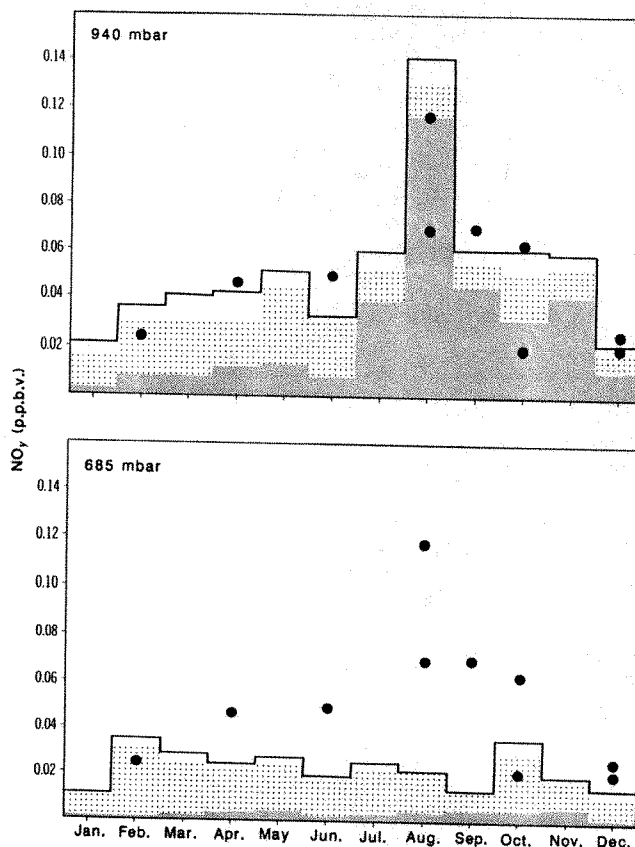


FIG. 1 Ten-day averages of soluble-nitrogen measurements at Mauna Loa Observatory compared with simulated monthly average mixing ratios of  $\text{NO}_x$  in the 940-mbar and 685-mbar grid boxes located over Hawaii. The dark shaded portion represents the simulated contribution of the US source, and the lighter shading represents the contribution from Asia (in our model a source region bounded on the south by the Equator, on the north by  $60^\circ \text{N}$  latitude, on the west by  $50^\circ \text{E}$  longitude and on the east by  $150^\circ \text{E}$  longitude) and the white area under the solid line represents the contribution from the rest of the globe, principally Mexico, Central America and Europe. The observations are represented by large black dots.

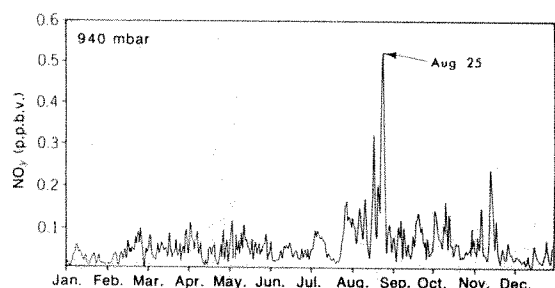


FIG. 2 The  $\text{NO}_y$  time series at the 940-mbar level in the vicinity of Hawaii constructed with instantaneous values taken every six hours from the simulation for a global source of combustion nitrogen.

synoptic analysis were used to identify this complex transport process (W.J.M., manuscript in preparation).

When using observed wind data, vertical velocity is not available and one must approximate atmospheric motion by assuming that either pressure or potential temperature remains constant. Such approaches can be misleading. Robinson and Harris<sup>11</sup> use observed winds to calculate approximate trajectories on constant-pressure surfaces for a number of actual transport events at Mauna Loa Observatory in August. Finding that none of the 700-mbar back-trajectories from Hawaii returned to the United States and none of the 850-mbar trajectories from the United States approached Hawaii, they conclude that US emissions are not involved<sup>11</sup>. We believe that the actual summertime transport events are as complex as those simulated by the model and cannot be represented by trajectories on constant-pressure surfaces.

The transport of  $\text{NO}_y$  from Asia is composed of a large number of relatively small ( $\sim 0.1$  p.p.b.v.) events. Asian dust, which also has surface sources and is removed by precipitation, may serve as a surrogate for Asian emissions. The dust has been observed throughout the central Pacific, including Mauna Loa, and has a spring maximum<sup>12,13</sup>. Nitrogen emissions from Asia are expected to follow a similar transport path. To reach Hawaii in the subtropics, the Asian emissions must first be lifted into the free troposphere by storms and carried eastward and north-eastward over the central Pacific. Then the  $\text{NO}_y$  must be caught in descending air, transported anticyclonically (clockwise) towards the Equator and carried far enough south to be caught in the low-level easterly flow. Such paths have been determined for actual springtime Asian dust events<sup>14</sup> and for our simulated springtime transport of Asian  $\text{NO}_y$ . Both the lifting over Asia and the descent over the Pacific occur at a range of levels in the troposphere. As a result, the model's Asian emissions are dispersed throughout the lower troposphere. In contrast, the transport from North America is descending all the way to Hawaii in the stable flow of the Pacific subtropical high and should arrive just above the maritime boundary layer.

Based on these results, other model studies<sup>9</sup>, observations<sup>12,13</sup> and actual climatology<sup>10</sup>, we conclude that the maximum in soluble nitrogen observed at Mauna Loa Observatory in the summer and autumn is a result of US fossil-fuel combustion. It travels to Hawaii in stable descending air and is carried up to the observatory by the complex flow associated with the mountain orography. Springtime measurements in the vicinity of Hawaii, but away from the influence of the island's orography, should find episodes of elevated  $\text{NO}_y$  (mainly from Asia) up into the middle troposphere, while summertime measurements should find episodes of elevated  $\text{NO}_y$  concentrated in shallow layers just above the maritime boundary layer. □

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## Synthesis of bulk superconducting $\text{YBa}_2\text{Cu}_3\text{O}_x$ at one atmosphere oxygen pressure

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THE well-known 90-K superconductor  $\text{YBa}_2\text{Cu}_3\text{O}_7$  ('123') is the first ( $n=0$ ) member of a homologous series of compounds with the general formula  $\text{Y}_2\text{Ba}_4\text{Cu}_{6+n}\text{O}_{14+n}$ . These compounds combine layers of copper–oxygen pyramids with single and/or double copper–oxygen chains. The  $n=2$  member,  $\text{YBa}_2\text{Cu}_4\text{O}_8$  ('124'), with double Cu–O chains, and the  $n=1$  ytterbium analogue,  $\text{Yb}_2\text{Ba}_4\text{Cu}_7\text{O}_{15}$  ('247'), which mixes single and double chains, were first observed as intergrowths in bulk 123 by electron microscopy<sup>1,11</sup>. The 124 phase was then synthesized as a majority phase in thin films<sup>2,3,12</sup>, and its crystal structure was determined<sup>4</sup> and found to be in agreement with the model proposed from microscopy. An important advance in the synthesis of bulk materials, the result of extensive pressure–temperature phase equilibria studies, was the isolation of 124 and 247 at oxygen pressures of  $>200$  atm, and detailed determinations of their crystal structures (see, for example, refs 5–9). High-pressure studies have also shown that the 124 phase could be made with many rare-earth elements<sup>13</sup>. Here we report the synthesis of the 124 phase in pure bulk form by a novel synthetic route in a flowing oxygen stream at 1 atm pressure. This technique allows  $\text{YBa}_2\text{Cu}_4\text{O}_8$  to be synthesized without specialized equipment, will make it generally available for study of its normal and superconducting properties, and will make possible more extensive comparisons with  $\text{YBa}_2\text{Cu}_3\text{O}_7$  and other high- $T_c$  superconductors. Our results suggest that 124 is the thermodynamically stable phase at low temperatures and 1 atm pressure, under oxidizing conditions; the usual inability to synthesize it in these conditions is probably due to the limitations of reaction kinetics.

The synthesis is a two-step process. In the first step, starting materials are mixed in the correct stoichiometric proportion and heated very slowly to 750 °C in dense, oversized  $\text{Al}_2\text{O}_3$  crucibles. They are then allowed to react for 16–24 h. All heating, soaking and cooling is carried out in flowing  $\text{O}_2$ . Best results are obtained when an intermediate mixing-and-grinding step is performed after the first few hours of reaction at 750 °C. Different combinations of starting materials yield slightly different results. We investigated three different sets of starting materials: (1)  $\text{Y}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$ ,  $\text{Ba}(\text{NO}_3)_2$  and  $\text{Cu}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$ ; (2)  $\text{Y}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$ ,  $\text{Ba}(\text{NO}_3)_2$  and  $\text{CuO}$ ; and (3)  $\text{Y}_2\text{O}_3$ ,  $\text{Ba}(\text{NO}_3)_2$  and  $\text{CuO}$ . The first of these, with all materials present as nitrates, always yielded the most phase-pure materials, although 124 did occur as the major phase for all three sets of starting materials. As it was necessary to use hydrated nitrates, the amount of water in the starting materials was determined directly by weight loss on decomposition to the simple oxides, which indicated that



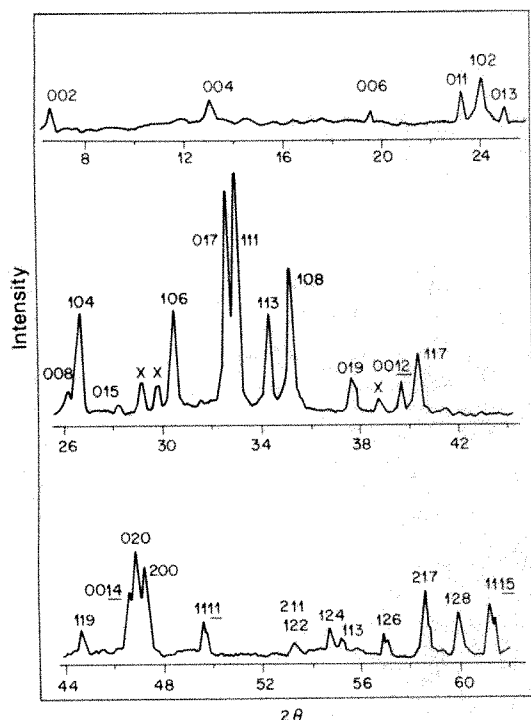


FIG. 1 Powder X-ray diffraction pattern of  $\text{YBa}_2\text{Cu}_4\text{O}_8$  prepared using a sodium carbonate catalyst. Peaks are indexed on an A-centred orthorhombic cell with lattice parameters  $a = 3.846$ ,  $b = 3.871$ ,  $c = 27.262$  Å. Impurity peaks are marked with crosses.

our starting materials were  $\text{Y}(\text{NO}_3)_3 \cdot 5.8\text{H}_2\text{O}$  and  $\text{Cu}(\text{NO}_3)_2 \cdot 3.3\text{H}_2\text{O}$ .

In the second step, the pre-reacted powder, after grinding, is mixed with an approximately equal volume of alkali carbonate powder. The alkali carbonate acts to catalytically enhance the reaction rate. For the synthesis of 124, either  $\text{Na}_2\text{CO}_3$  or  $\text{K}_2\text{CO}_3$  can be used for this purpose, but  $\text{Na}_2\text{CO}_3$  yields better phase purity. The 124-carbonate mixture is ground, placed in silver-foil packets or crucibles and heated at  $800^\circ\text{C}$  in flowing  $\text{O}_2$  for 3 days. The carbonates are not molten at this temperature.  $\text{YBa}_2\text{Cu}_4\text{O}_8$  is the main phase present after just one day, but the phase purity improves for longer reaction times. If the first reaction step is not carried out,  $\text{BaCO}_3$  forms in the initial stages of the carbonate reaction and the desired phase cannot be made. The 124 phase can also be made, albeit less reliably, at  $825^\circ\text{C}$ ; but by  $850^\circ\text{C}$ ,  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  forms as a major impurity phase, which is consistent with the metastability of  $\text{YBa}_2\text{Cu}_4\text{O}_8$  described in ref. 8. We are able to make 124 as the majority phase at temperatures as low as  $700^\circ\text{C}$  by this process. At the lowest temperatures, approximately equal mixtures of  $\text{K}_2\text{CO}_3$  and  $\text{Na}_2\text{CO}_3$  function as the most effective catalysts, as such a mixture is closer to its melting point during the reaction than is either of the pure carbonates. After reaction, the products are washed briefly (15 min) in water to remove excess alkali carbonate and dried by gentle heating in air. The product is a fine-grained powder.

A representative powder X-ray diffraction pattern of the material produced by this technique is shown in Fig. 1. The peaks have been indexed by position and by the intensities calculated from the published crystal structure. The materials made by this method, although in general not perfectly single-phase, are at least as phase-pure as materials prepared at high oxygen pressures. Three impurity peaks are present in Fig. 1 with intensities of  $\sim 10\%$  of the main peak intensity, at  $d$  values (inter-planar spacings) of  $2.83$  Å,  $2.92$  Å and  $2.32$  Å ( $\text{CuO}$ ). For the  $\text{Na}_2\text{CO}_3$ -catalysed formation of 124 at  $800^\circ\text{C}$ , the 123 phase is not usually present as an impurity, but it may be present if  $\text{K}_2\text{CO}_3$  is used as the catalyst. Improved phase purity may be

achieved by careful control of the composition and uniformity of the hydrated-nitrate starting materials, or of the initial stages of the dehydration process, which involves the evolution of a large amount of gas from a mixture which is apparently molten at very low temperatures ( $100$ – $200^\circ\text{C}$ ). Least-squares refinement of the crystallographic unit cell from 17 of the powder diffraction peaks between  $2\theta$  values of  $30^\circ$  and  $62^\circ$ , consistent with an A-centred orthorhombic cell, yields lattice parameters  $a = 3.8457(9)$ ,  $b = 3.8711(9)$  and  $c = 27.2616(61)$  Å, in excellent agreement with the orthorhombic cell parameters published previously<sup>9</sup>.

Figure 2 shows the results of d.c. magnetization measurements on  $\text{YBa}_2\text{Cu}_4\text{O}_8$  prepared at  $800^\circ\text{C}$  in  $\text{O}_2$  with  $\text{Na}_2\text{CO}_3$  as catalyst. The sample is a pellet pressed from the fine-powder product, but not sintered. The measurements were made using an SHE squid magnetometer in a field of  $25$  Oe. Flux expulsion below  $T_c$  (on field-cooling) corresponds to  $\sim 60\%$  of that expected for a perfect diamagnet. This is a large apparent superconducting volume fraction for a pressed-powder sample, and actually represents a conservative lower limit for the true fraction of superconducting material, because flux trapping in pores may in general limit the flux expulsion significantly, as is evident here in the slowly increasing diamagnetism measured at temperatures well below the superconducting transition. The transition temperature ( $77$  K) is within the range of temperatures reported previously but is somewhat lower than the maximum of  $80$ – $81$  K reported for  $\text{YBa}_2\text{Cu}_4\text{O}_8$  prepared at high oxygen pressures. The reason for this variation is not yet clear, as the compound has been reported to be stoichiometric in composition for all components, including oxygen<sup>5</sup>. Annealing our material at  $450^\circ\text{C}$  in flowing  $\text{O}_2$  for 16 h did not increase  $T_c$  substantially. There is no diamagnetism above  $77$  K, indicating, as did the powder X-ray diffraction data, that  $\text{YBa}_2\text{Cu}_3\text{O}_7$  is not present as an impurity under these synthetic conditions.

We have also measured the temperature dependence of the crystallographic cell parameters between  $30$  and  $320$  K. We were particularly interested to see whether structural anomalies occur near  $240$  K, where kinks in the resistance and Hall coefficient have been observed in thin-film samples (ref. 10 and S. Martin *et al.*, manuscript in preparation). The temperature dependence was measured by taking  $\theta$ – $2\theta$  scans at moderately high angles ( $56$ – $63^\circ 2\theta$ ) and fitting to the positions of six peaks of the powder pattern. The instrumental resolution was  $\sim 0.028$  Å<sup>–1</sup> full width at half maximum. The results of the fits (Fig. 3) do not indicate any anomalies in the lattice parameters in the range  $30$ – $320$  K. In addition, we observed no indication of a lower symmetry in

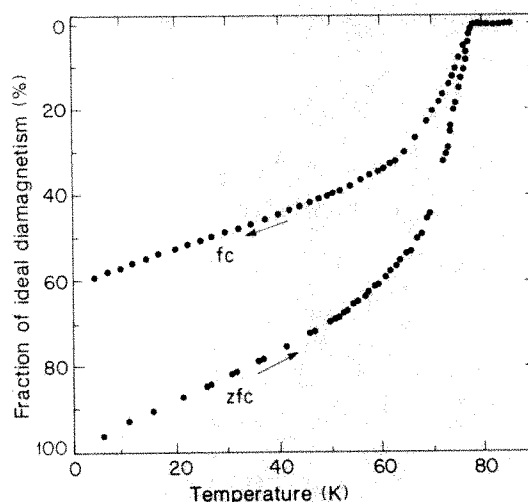


FIG. 2 Temperature dependence of d.c. magnetization for a pressed-powder compact of  $\text{YBa}_2\text{Cu}_4\text{O}_8$ , measured in a d.c. field of  $25$  Oe. Zero-field cooled (zfc) and field-cooled (fc) measurements are shown.

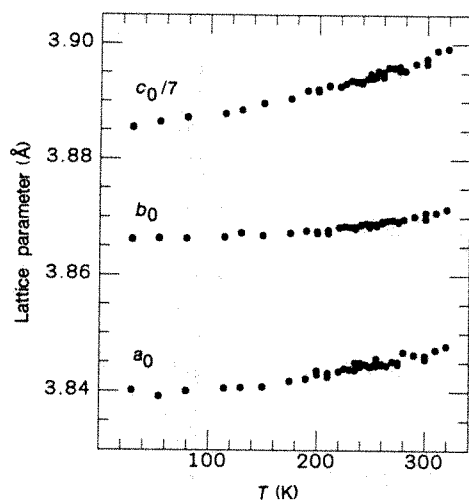


FIG. 3 Temperature dependence of the crystallographic unit-cell parameters for  $\text{YBa}_2\text{Cu}_4\text{O}_8$  between 30 and 320 K.

the powder scans at 30 K, nor were extra peaks observed that would indicate a multiplication of the unit-cell volume.

In conclusion, we have shown that the compound  $\text{YBa}_2\text{Cu}_4\text{O}_8$  can be prepared as a bulk phase at ambient pressure in a novel multi-step process which employs an alkali carbonate to enhance the reaction rate. Special care must be taken in the selection of the starting materials, the order of the reaction steps, selection of the container materials, the temperature of reaction, and the use of a non-detrimental reaction-rate enhancer. The process described here yields high-quality 124 without specialized equipment and will allow considerably more research to be performed on its physical properties. Finally, we note that our attempts to synthesize  $\text{Y}_2\text{Ba}_4\text{Cu}_7\text{O}_{15}$  by the same method were not successful. □

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## Separation of paramagnetic and diamagnetic molecules using high- $T_c$ superconducting ceramics

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WITH the advent of high-temperature superconductivity, the search is on for applications of these materials. Here we outline a new method for the separation of paramagnetic and diamagnetic molecules using the repulsive interaction between a magnetic dipole and a micro-cavity in a ceramic material which is in the supercon-

TABLE 1  $\text{O}_2/\text{N}_2$  ratio in out-flow gas through  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  and a  $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet as a function of temperature

$\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ pellet		$\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet	
$\text{O}_2/\text{N}_2$	$T$ (K)	$\text{O}_2/\text{N}_2$	$T$ (K)
0.04	85.3	0.26	84.8
0.05	85.6	0.27	85.0
0.05	86.2	0.26	85.2
0.05	86.4	0.27	85.6
0.08	86.6	0.27	86.2
0.10	87.6	0.27	87.5

ducting state. We present experiments of the permeation of air through  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  and through  $\text{Bi}_{1.8}\text{Pb}_{0.2}\text{Ca}_2\text{Sr}_2\text{Cu}_3\text{O}_x$  ceramic pellets. These experiments show the preferential permeability of nitrogen compared with that of oxygen in the diamagnetic state of the superconductors below the critical transition temperature,  $T_c$ . This effect is quenched by the application of a large enough external magnetic field. As far as we know it is the first application of the porosity of the ceramic superconductors.

The energy of interaction ( $E$ ) of a magnetic dipole ( $m$ ) and a superconducting wall is easily calculated by the mirror principle<sup>1</sup> (see Fig. 1). This interaction is given by:

$$E = \frac{\bar{m}_1 \bar{m}_2}{r^3} - \frac{3(\bar{m}_1 \bar{r})(\bar{m}_2 \bar{r})}{r^5} = \frac{m^2}{r^3} \{\cos \varepsilon - 3 \cos \theta_1 \cos \theta_2\} \quad (1)$$

where  $\bar{m}_2$  is the mirror image of  $\bar{m}_1$ , and  $\theta_1 + \theta_2 = 180^\circ$ . The thermal tumbling of  $\bar{m}$ , when averaged over all orientations  $\theta$ , gives  $\langle E \rangle = \frac{2}{3} (m^2/r^3)$ . Suppose we enclose the tumbling magnetic dipole in the centre of a cubic cavity: at what distance from the walls of the cavity is the repulsive interaction of the superconducting walls equal to the thermal kinetic energy of the elementary magnetic moment? We have  $6\langle E \rangle = \frac{2}{3} kT$ , and thus  $r = (6m^2/kT)^{1/3}$ . We can now substitute  $m^2$  by the paramagnetic susceptibility  $\chi(T) = m^2 N/3kT$ , where  $N$  is Avogadro's number, so that

$$r = \left( \frac{18\chi(T)}{N} \right)^{1/3} \quad (2)$$

From equation (2) we can estimate the effective distance  $r$  at which the repulsive interaction between the molecular magnetic moment and the superconducting diamagnetic enclosure will strongly affect the thermal diffusivity of the magnetic dipole. Let us compare the relevant size parameters for oxygen (a paramagnetic molecule) to those of diamagnetic nitrogen. The effective molecular diameter of oxygen, as derived from the Einstein-Stokes equation using the diffusion constant of  $\text{O}_2$  in ethanol<sup>2</sup>, is 1.67 Å, and the same calculation for  $\text{N}_2$  in carbon tetrachloride yields 1.24 Å; in comparison,  $2r$  for oxygen (using  $\chi(91 \text{ K}) = 1.08 \times 10^{-2} \text{ ml mol}^{-1}$  (ref. 3)) is 1.37 Å. Nitrogen, on the other hand, is diamagnetic,  $\chi(300 \text{ K}) = -12 \times 10^{-6} \text{ ml mol}^{-1}$ . We see that the diameter,  $2r$ , is comparable with the effective diameter as calculated from the Einstein-Stokes equation. The Pauling dimensions for  $\text{O}_2$  are 2.8 Å in width and 3.9 Å in length, with a kinetic diameter  $\sigma = 3.46$  Å. The corresponding values for nitrogen are 3.0 Å, 4.1 Å and 3.64 Å respectively<sup>4</sup>. The apparently small difference in the kinetic diameters of these two gases is thought to be one of the main reasons why zeolites and glassy polymers show preferential sorption and permeation of oxygen relative to nitrogen<sup>5,6</sup>. We expect therefore that the repulsive interaction will increase the effective kinetic diameter of oxygen. Thus if air is diffused through a superconducting ceramic material at  $T < T_c$ , we expect preferential permeability of nitrogen, provided that the average pore diameter is low enough ( $\sim 10$  Å). We examine, as an example, the possibility of preferential permeability of nitrogen over that of oxygen

through  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  in the diamagnetic state. Such a separation process is expected to take place for a diffusion-controlled permeation of air through the superconducting pellet in the temperature range in which the diamagnetic susceptibility is fully developed. (The Meissner onset temperature in our samples was 102 K and the diamagnetic effect was fully developed at  $\sim 77$  K.) The permeation experiment was therefore performed using a low-pressure gradient of 50 kPa starting at the lowest temperature possible, just above the dew point of air (81.3 K at 100 kPa (ref. 7)). Below the dew point, the results of the permeation experiments are obscured by the gas-liquid equilibria of oxygen and nitrogen.

We conducted an experiment (see Fig. 2) in which dry air was passed over and diffused through a  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  pellet, 13 mm in diameter and 1 mm thick, mounted in a brass block cooled by liquid nitrogen. The composition of the outflow gas ( $\sim 0.4$  ml  $\text{min}^{-1}$  at 26 °C) was analysed by a gas chromatograph in real time. The resulting run, measured at a heating rate of 0.1 K  $\text{min}^{-1}$ , in the temperature range between 82 K and 92 K, is shown in Fig. 3a. The critical transition temperature ( $T_c$ ) for this pellet was  $92 \pm 0.5$  K, and the temperature dependence of the resistance is shown in Fig. 4a. This sample was quite porous, its specific density  $\rho = 5.64$  g  $\text{cm}^{-3}$  being comparable with the specific theoretical density  $\rho_{\text{th}} = 6.4$  g  $\text{cm}^{-3}$ .

In a control experiment a  $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet was prepared by a fast quench of a  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  pellet from 900 °C to liquid-nitrogen temperatures. This sample did not exhibit the Meissner effect and a plot of resistance against temperature is shown in Fig. 4b. This pellet was similar in density and micro-grain structure to the superconducting sample used above. It is apparent from comparison of the plots in Fig. 3a and b that

the superconducting sample does impede the mobility of  $\text{O}_2$  for  $T < 83$  K, and indeed shows preferential permeability for nitrogen in comparison with that of the control. This trend is followed by a sharp desorption of the trapped oxygen for  $T \approx 85$  K; the permeate stream composition subsequently tails off to the  $\text{O}_2/\text{N}_2$  ratio of 0.268, which is that observed in air.

We suggest that the experimental results may be interpreted as the manifestation of preferential permeability of nitrogen over that of oxygen in a temperature range in which the diamagnetism of the ceramic material is strong, followed by desorption of oxygen upon the increase of the magnetic susceptibility in the pellet.

In another experiment the relative permeation of  $\text{O}_2$  against  $\text{N}_2$  was measured through a 9-mm-thick  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  pellet and through a  $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich sample of the same geometry using a pressure gradient of 80 kPa and a flow rate of  $\sim 0.2$  ml  $\text{min}^{-1}$ . The results, in the temperature range 85–88 K measured at a heating rate of 0.1 K  $\text{min}^{-1}$ , are presented in Table 1. In this experiment high selectivity is observed, with  $(\text{O}_2/\text{N}_2)_{\text{air}}/(\text{O}_2/\text{N}_2)_{\text{permeate}} \approx 5$ .

We expect that the application of a magnetic field,  $H > H_c$ , to the diamagnetic membrane in the superconducting state will quench the selectivity for nitrogen against oxygen permeation that is observed for  $T < T_c$  and  $H = 0$ .  $\text{Bi}_{1.8}\text{Pb}_{0.2}\text{Ca}_2\text{Sr}_2\text{Cu}_3\text{O}_x$ , a ceramic with a high  $T_c$  ( $111 \pm 1$  K) and low  $H_c$  ( $< 100$  Oe at 4.2 K), was prepared to test this prediction. The permeation experiment was conducted through a 2-mm-thick pellet using a pressure gradient of 50 kPa, a flow rate of 0.4 ml  $\text{min}^{-1}$  and a

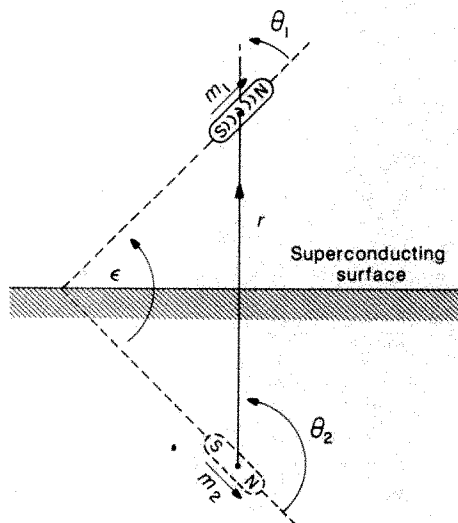


FIG. 1 The magnetic moment  $\vec{m}_1$  and its mirror image  $\vec{m}_2$  in the superconducting wall.

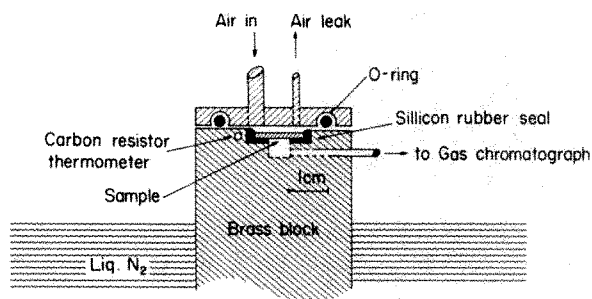


FIG. 2 Experimental set-up for measurement of  $\text{O}_2$  and  $\text{N}_2$  permeabilities through ceramic pellets at low temperature.

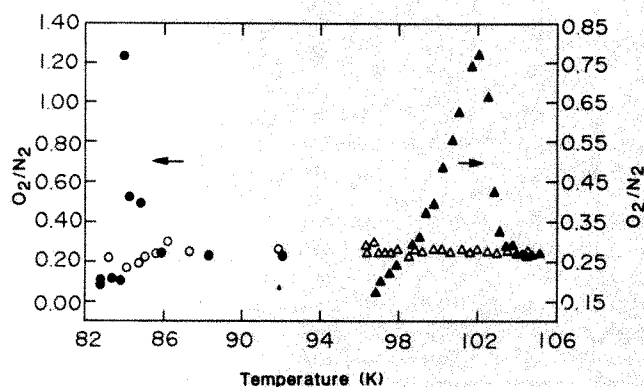


FIG. 3  $\text{O}_2/\text{N}_2$  ratio in the out-flow gas as a function of temperature, for  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  (●), a  $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet (○), and  $\text{Bi}_{1.8}\text{Pb}_{0.2}\text{Ca}_2\text{Sr}_2\text{Cu}_3\text{O}_x$  in a field of  $H = 0$  (▲) and  $H = 220$  Oe (△).

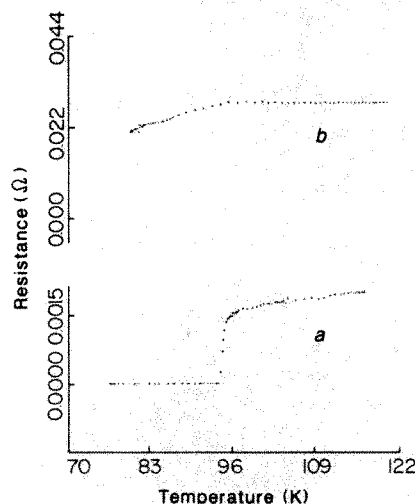


FIG. 4 Resistance against temperature for a, a  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  pellet, and b, a  $\text{YBa}_2\text{Cu}_3\text{O}_6$ -rich pellet.



heating rate of  $0.1 \text{ K min}^{-1}$ . Figure 3c, d shows the results for a magnetic field ( $H=0$  and  $H=220 \text{ Oe}$ ) applied perpendicularly to the surface of the pellet. The measured selectivity is shifted to a higher temperature range than that observed for  $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$  (see Fig. 3a, b) as is the desorption peak, which is at  $\sim 102 \text{ K}$  for the zero-field run. A field of  $220 \text{ Oe}$  is large enough to quench the selectivity effect as the magnetic flux penetrates the diamagnetic membrane. (Note that at  $\sim 100 \text{ K}$ ,  $H_{c1} < 20 \text{ Oe}$ .)  $\square$

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## Different behaviour of platinum in the Indian and Pacific Oceans

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THERE is little known about the chemical behaviour of the platinum-group metals in the ocean. Here we have used cathodic stripping voltammetry<sup>1</sup> to determine the concentration of dissolved platinum in the water column of the Indian Ocean. The range of Pt values obtained ( $0.2\text{--}1.6 \text{ pM}$ ) is similar to that found previously in the Pacific Ocean<sup>2-4</sup>. But unlike the earlier Pacific Ocean investigations which depicted platinum as having nutrient-like behaviour, our data show that in the Indian Ocean the element is scavenged down the water column and so is depleted with depth. This is consistent with the predominant oxidation state of platinum in oxygenated sea water being Pt(IV), which is also predicted from thermodynamic considerations and the association of platinum with manganese nodules in sediments<sup>5</sup>. The data show that platinum co-varies in the water column with dissolved manganese, the concentration of Pt being lower than Mn by a factor of about 600. But the residence time of Pt in the oceans is longer than that of Mn, as revealed by a Pt/Mn ratio in the Indian Ocean that is 300 times higher than in crustal rock.

Seawater samples were obtained at  $27^\circ 00' \text{ S}$ ,  $56^\circ 58' \text{ E}$  (station CD 1504) and  $06^\circ 09' \text{ S}$ ,  $50^\circ 54' \text{ E}$  (station CD 1507) during cruise 15 of the RRS *Charles Darwin* in the Indian Ocean<sup>5</sup>. Clean laboratory and sampling procedures were used to avoid sample contamination<sup>6</sup>. The samples were collected using 10-litre polytetrafluoroethylene-lined Go-Flo bottles mounted on a rosette with conductivity, temperature and depth measuring instrumentation. The sea water was immediately filtered under pressure through acid-washed  $0.4 \mu\text{m}$  'Nucleopore' filters in teflon filter holders into acid-washed high-density polyethylene bottles and stored frozen. Before analysis, samples were thawed and acidified to pH 2 with HCl (Aristar grade), and a period of at least a week was allowed to elapse for desorption from the bottle walls and dissolution of any particulates formed in the process of freezing and thawing. The samples were then irradiated with ultraviolet light for 6–8 hours to break down

organic material such as surfactants and complexing ligands.

Dissolved platinum was determined by cathodic stripping voltammetry<sup>1</sup>. When sulphuric acid ( $0.5 \text{ M}$ ), hydrazine ( $0.0015\%$ ) and formaldehyde ( $0.012\%$ ) were added to a  $10 \text{ ml}$  aliquot of each sample, the formazone produced formed a complex with Pt(II). A small constant fraction of this complex is adsorbed on a hanging mercury drop electrode during a preconcentration stage that lasts for 5 min and catalyses the formation of hydrogen when the electrode potential is scanned in a negative direction. The reduction current associated with this hydrogen formation is linearly related to the combined Pt(II) and Pt(IV) concentration<sup>1</sup>. Supplementary data such as the temperature, salinity, amounts of dissolved oxygen, nutrients and other metals were obtained during the cruise and are described elsewhere<sup>5,6</sup>.

The vertical distribution of platinum, manganese and salinity at stations CD 1504 and CD 1507 is shown in Figs 1 and 2. Depletion with depth is shown in both Pt profiles and for Mn. The drop in the Pt concentrations in the upper water column occurs at a depth that is shallower at station CD 1507 than at station CD 1504. This is consistent with upwelling at station CD 1507 and with downwelling at station CD 1504, as evidenced by the shape of the nutrient/depth profiles.

Some co-variation of Pt and Mn is apparent from a linear regression of the Pt concentration (pM) as a function of the Mn concentration (nM) ( $[\text{Pt}] = a[\text{Mn}] + b$ ), where  $a = 1.06 \pm 0.28$  and  $b = 0.30 \pm 0.31$ . The standard deviations are quite large because of the variation in Mn concentrations in the deep waters (the Mn concentrations were almost at the limit of detection) and because of a scarcity of data for the upper water column, where most of the change in the platinum concentration occurs. Analysis of a number of samples without prior ultraviolet photolysis showed that interference by dissolved organic material, presumably consisting of surface-active as well as chelating compounds, was particularly strong in the upper water column. But this interference was removed in the ultraviolet-photolysis step, as was apparent from the voltammetric sensitivity (the ratio of the reduction current over the platinum concentration), which was constant for samples from all depths.

The ratio of crustal Mn to Pt is  $\sim 2 \times 10^5$  (crustal data from Govett<sup>7</sup>), whereas the ratio of Mn to Pt in sea water is  $\sim 600$ . Thus platinum is significantly enriched relative to manganese in sea water compared with its crustal value, which suggests that platinum is not as efficiently scavenged as manganese, and has a longer residence time. Calculation of the inorganic speciation of Pt(II) in sea water using published stability constants<sup>8</sup> shows that it occurs largely (98%) as the  $\text{PtCl}_4^{2-}$  complex (which is an anion, and would tend to behave conservatively), the rest being made up of  $\text{PtCl}_3^-$  complexes. The overall  $\alpha$ -coefficient<sup>9</sup> of complexation of Pt(II),  $\alpha_{\text{Pt}} = [\text{Pt}_T]/[\text{Pt}^{2+}]$ , where  $[\text{Pt}_T]$  is

TABLE 1 The concentration of platinum in the Western Indian Ocean

Station CD 1504		Station CD 1507	
Depth (m)	Pt (pM)	Depth (m)	Pt (pM)
25	1.3	5	1.6
77	1.1	45	1.3
152	1.6	105	1.1
500	0.45	200	0.80
799	0.80	650	0.83
1,080	0.20	880	0.30
1,750	0.29	1,506	0.33
2,451	0.17	2,003	0.41
3,002	0.30	2,850	0.51
3,749	0.22	3,451	0.37
4,550	0.21	4,050	0.43
4,926	0.29	4,650	0.39
		4,855	0.36

The standard deviation of the Pt concentrations is  $\pm 10\%$ .

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the total Pt(II) concentration, is calculated from  $\alpha_{\text{Pt}} = 1 + \sum K_{\text{PtCl}_i} [\text{Cl}^-]^i$  (hydrolysis of  $\text{Pt}^{2+}$  is negligible in the presence of  $\text{Cl}^-$ ) and has a value of  $10^{12}$ . The much greater value for  $\alpha_{\text{Pt}}$  than for  $\alpha_{\text{Mn}}$  ( $\sim 2$ ; ref. 10) may explain the enrichment of Pt in sea water relative to Mn, as uncomplexed cationic Mn(II) would tend to be scavenged more easily than the anionic chloride species of Pt(II).

The profiles for Pt in the Indian Ocean are quite different from those obtained in earlier studies from the Pacific, which showed that the element behaved like a nutrient<sup>2-4</sup> (a comparative profile for Pt displaying this behaviour in the Pacific Ocean is illustrated in Fig. 2c). But because Pt is enriched relative to Pd in ferromanganese nodules, and because of the greater stability of Pt(IV) relative to Pd(IV), it has been proposed that Pt might also be subject to oxidative removal<sup>2,3</sup>. The reduction of Pt(IV) to Pt(II) in an electrolyte containing chloride ions (sea water) is described by<sup>11</sup>  $\text{PtCl}_6^{2-} + 2\text{e}^- \rightleftharpoons \text{PtCl}_4^{2-} + 2\text{Cl}^-$ , and has a value of 0.68 V for  $E^0$ . According to the Nernst equation, the ratio of Pt(II)/Pt(IV) in sea water depends on the potential,  $E$ ,

as  $E = 0.67 - 0.03 \log ([\text{PtCl}_4^{2-}]/[\text{PtCl}_6^{2-}])$ . The redox potential,  $E_{\text{H}}$ , of oxygenated water is determined by the  $\text{O}_2/\text{H}_2\text{O}$  redox couple and lies at  $E_{\text{H}} = 0.75$  V, whereas in surface sea water in the presence of  $10^{-7}$  M hydrogen peroxide<sup>12</sup> it is controlled by the  $\text{O}_2/\text{H}_2\text{O}_2$  couple and  $E_{\text{H}} = 0.40$  V. The calculated equilibrium ratio of Pt(II)/Pt(IV) amounts to  $10^9$  in surface waters, whereas in deeper oxygenated waters it is 0.002. Therefore the thermodynamically preferred oxidation state of Pt is Pt(IV) in most of the oceanic water column, whereas in surface waters it is Pt(II). The scavenged behaviour of Pt in the Indian Ocean is consistent with this thermodynamic prediction. But perhaps it is not consistent with the rather long residence time of Pt which would be about 300 times longer than that of Mn on the basis of the Pt/Mn ratio in the crust as compared with the ratio in seawater.

The deep-water concentration of Pt in the Indian Ocean is lower than in the Pacific, whereas the concentration in the upper water column is greater, but the cause of this difference is not clear. Perhaps it results from small differences in the  $E_{\text{H}}$  arising from variations in the dissolved oxygen concentration or of

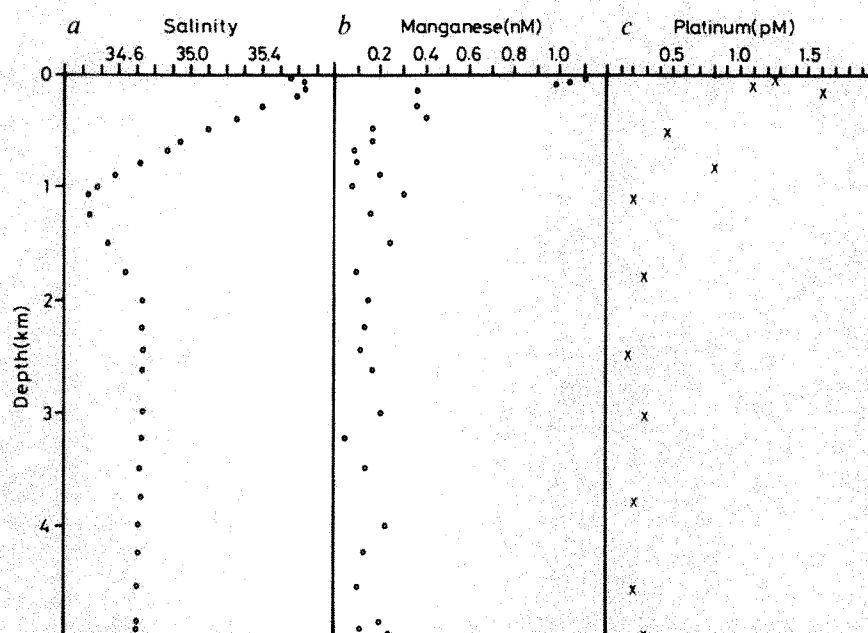


FIG. 1 Vertical distribution of a, salinity b, manganese (from N. H. Morley, P. Statham & J. D. Burton, manuscript in preparation) and c, platinum in Station CD 1504.

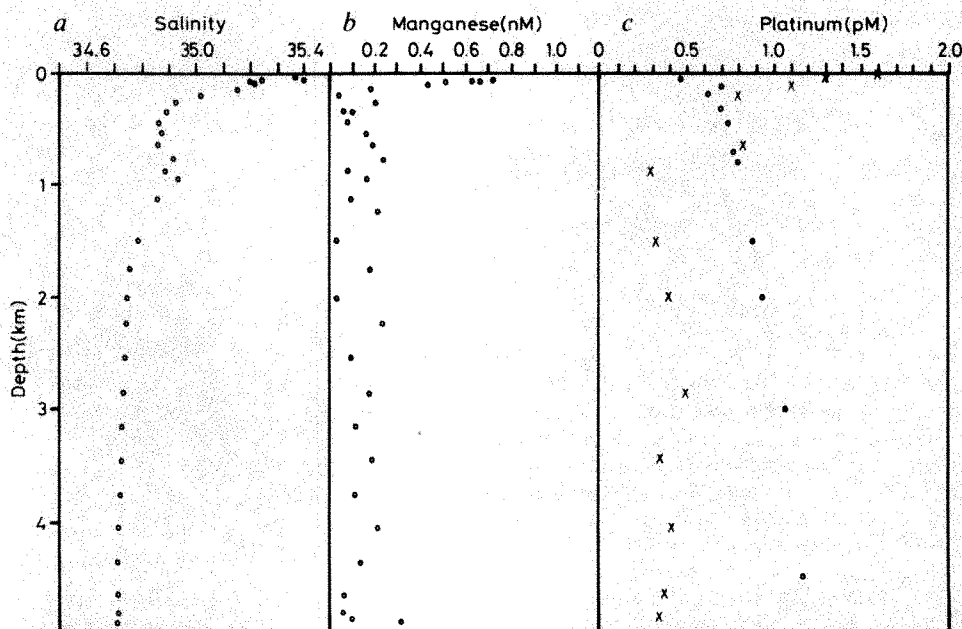


FIG. 2 Vertical distribution of a, salinity b, manganese (from N. H. Morley, P. Statham & J. D. Burton, manuscript in preparation) and c, platinum in Station CD 1507; in c, the platinum concentration in the Pacific Ocean<sup>3</sup> (circles) is shown along that in the Indian Ocean (crosses).

localized inputs from hydrothermal (in the Pacific deep waters) or eolian (surface Indian Ocean waters) origin. The possibility cannot be discounted that the difference is caused by an experimental artefact, perhaps as a result of organic trace metal speciation. For instance, it is possible that the radioactive tracer<sup>3</sup> that was added to samples before Pt determination by GF-AAS, to verify the accumulation efficiency of the anion-exchange resin, did not reach equilibrium with organically complexed Pt. This is unlikely, as the samples were acidified to low pH; nevertheless, very stable metal-organic complexes such as of Cu and Ni occur in sea water<sup>13,14</sup>, and a small amount of organically complexed copper is stable in acidified samples<sup>15</sup>. Furthermore, it is not impossible that complexation of Pt(II) by natural ligands is much stronger than with Cu(II) or Ni(II) if we consider the high value for  $\alpha_{Pt}$  in sea water and the large shift in the reduction potential ( $>1$  V, equivalent to a value for  $\log \alpha_{Pt-formazine} > 30$ ) on complexation of Pt(II) by formazone before voltammetric analysis<sup>1</sup>. Such organic complexing material, if present, should have been removed in the ultraviolet-photolysis step and would therefore not interfere with the voltammetric determination of Pt.

The interesting chemistry of platinum in the oceans should be investigated further if indeed the redox properties of Pt(IV)/Pt(II) and the strong complexation of Pt(II) can account for the observed differences in the Pt concentrations in the Pacific and Indian oceans. □

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## Oscillatory responses in cat visual cortex exhibit inter-columnar synchronization which reflects global stimulus properties

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A FUNDAMENTAL step in visual pattern recognition is the establishment of relations between spatially separate features. Recently, we have shown that neurons in the cat visual cortex have oscillatory responses in the range 40–60 Hz (refs 1, 2) which occur in synchrony for cells in a functional column and are tightly correlated with a local oscillatory field potential. This led us to hypothesize that the synchronization of oscillatory responses of spatially distributed, feature selective cells might be a way to establish relations between features in different parts of the visual field<sup>2,3</sup>. In support of this hypothesis, we demonstrate here that neurons in spatially

separate columns can synchronize their oscillatory responses. The synchronization has, on average, no phase difference, depends on the spatial separation and the orientation preference of the cells and is influenced by global stimulus properties.

We recorded multi-unit responses to appropriately oriented moving light bars simultaneously from 5 to 7 spatially separate sites in cortical area 17 of 13 adult cats. To determine the temporal relationship of the firing patterns recorded at two sites, we computed both the auto- and cross-correlation functions of the spike trains<sup>4,5</sup>. For 132 of 199 recording sites, the auto-correlation function of the responses was periodic, indicating that the neuronal responses were oscillatory. To establish an objective criterion for the occurrence of oscillatory responses, we fitted a damped sine wave (Gabor function) to the auto-correlograms. Responses were considered to be oscillatory when the fitted function had at least three peaks and when the amplitude of the sinusoidal modulation was significantly different from zero ( $P < 0.05$ ) and exceeded 10% of the amplitude of the cross-correlogram recomputed after shuffling the trial sequence by one stimulus period. The frequency of these oscillatory responses ranged from 40 to 60 Hz (mean,  $50 \pm 6$  Hz), was similar for different recording sites in the same animal and depended only slightly on stimulus configuration (orientation, direction)<sup>2</sup>. Of these 132 recordings, we selected 99 pairs in which oscillatory responses occurred simultaneously at two sites, and used these in cross-correlation analysis. Applying the same criteria used for auto-correlograms, 51 of the cross-correlograms had a significant correlation between the oscillatory responses.

Figure 1 illustrates a typical case in which neuronal responses were oscillatory and synchronized across spatially separate columns. Responses were recorded from five closely spaced sites near the representation of the area centralis of the retina. The receptive fields were overlapping but had different orientation preferences at adjacent sites. Stimulation with a light bar of  $112^\circ$  orientation evoked vigorous responses at sites 1, 3 and 5 but not at sites 2 and 4. As indicated by the periodic modulations of both auto- and cross-correlation functions, these responses were oscillatory and the oscillations were tightly correlated with zero phase difference. Changing the orientation of the stimulus to  $22^\circ$ , to maximize activation of the units at sites 2 and 4 produced synchronized oscillatory responses at these sites (data not shown). In all cases the correlations were abolished in the shuffled cross-correlogram<sup>4–11</sup>.

When the recording sites had a larger spatial separation ( $>2$  mm) the receptive fields were non-overlapping and could be stimulated independently. This enabled us to activate the

TABLE 1 Correlated oscillatory neuronal responses in area 17 as a function of spatial separation of recording sites and angular difference in preferred stimulus orientation

Angular difference of preferred orientation	Spatial separation	
	0.4–2.0 mm (overlapping fields)	2.0–7.0 mm (non-overlapping fields)
0–22°	90% (28/31)	54% (7/13)
45°	73% (8/11)	0% (0/8)
67–90°	44% (7/16)	25% (1/4)

Data were taken from a total of 99 cross-correlograms in which simultaneous oscillatory responses were recorded from two electrodes in area 17. Correlograms computed for responses at sites separated by 8–12 mm ( $n=16$ ) were excluded. The correlograms were classified into six categories based on the difference in orientation preference of the neurons at the two recording sites and whether or not the receptive fields were overlapping. The results are presented as the percentage of recordings showing oscillatory correlations. The numbers in parentheses correspond to the number of oscillatory correlations and the total number of response pairs analysed for that category, respectively. The correlograms which showed no periodic modulation (48 out of 99) showed either a single peak centred around a 0 ms time delay ( $n=11$ ) or a flat distribution ( $n=37$ ).



units at each site even if their preferred orientations differed and to determine more precisely if the extent of correlations depended on the similarity of orientation preferences. Figure 2 illustrates the typical case where oscillatory responses in remote columns were synchronized if their orientation preferences were similar but showed no fixed phase relationship when the orientation preferences differed.

In two individuals we recorded at two sites separated by 7 mm in which the receptive fields were non-overlapping, had the same orientation preference and were aligned colinearly. This enabled us to co-activate the units at both recording sites with a single long light bar, as well as with two short, independently moving stimuli (Fig. 3). In both cases, the stimuli evoked oscillatory responses at each site. When the short light bars were moved in opposite directions over the two receptive fields, the respective responses showed no phase locking. When the two stimuli were moved in the same direction, however, the oscillations became weakly synchronized and this synchronization was markedly enhanced in each case when the responses were evoked with a single long light bar that co-stimulated the two receptive fields. This suggests that synchronization depends on global features of the stimuli such as coherent motion and continuity, which are not reflected by the local responses alone.

The probability for the occurrence of phase locking depended both on the distance between recording sites and on the angular difference between preferred stimulus orientations (Table 1). There was no phase locking of the oscillatory responses when the electrodes were separated by 7–12 mm ( $n=16$ ). At intermediate distances of 2–7 mm, when the receptive fields of the recorded neurons were non-overlapping, phase locking occurred mainly between neuronal groups with similar orientation preferences. The same trend was observed for more closely spaced neurons (0.4–2.0 mm), which had overlapping receptive fields but in these cases phase locking was also observed for cells with different orientation preferences. Phase locking of oscillatory

responses typically occurred with a phase difference of 0 ms (32 out of 51) and the phase difference rarely exceeded  $\pm 3$  ms.

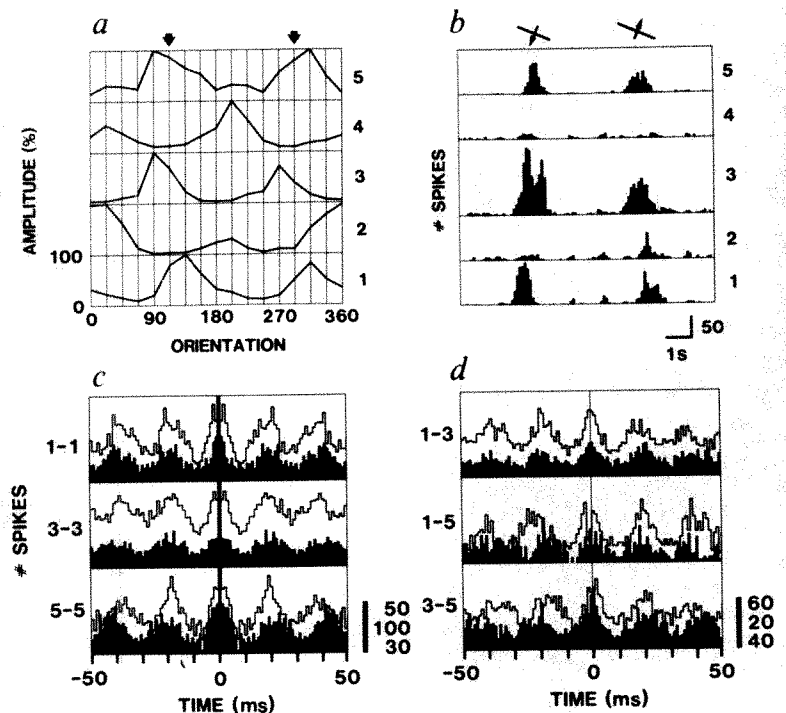
Our auto-correlation data confirm that the responses of a large fraction of cortical neurons are oscillatory and that these oscillations are synchronous for cells that are close enough together to be recorded with a single electrode<sup>1,2</sup>. Thus, we could take advantage of multi-unit recordings for the cross-correlation analysis, considerably increasing the number of events per unit time and allowing us to confine the analysis to short epochs. Correlations between the firing probabilities of neurons in the visual cortex have been described previously<sup>6–12</sup> and shown to be dependent on orientation preference<sup>6,9</sup> but only one study has provided evidence for oscillatory correlograms<sup>11</sup>. This relative lack of evidence for oscillatory responses in the cortex has several possible explanations. First, only a fraction of cortical neurons have oscillatory responses<sup>2</sup>; second, averaging procedures mask the oscillations because they are not phase-locked to the stimulus<sup>2</sup>; and third, previous studies may have excluded oscillatory activity because cross-correlograms of rhythmic responses were interpreted as misleading<sup>6,12</sup>.

The system of tangential intracortical connections<sup>13–19</sup>, or the reciprocal projections from other cortical areas<sup>20</sup> may provide the anatomical substrate for the synchronization of oscillatory responses between remote columns. Common input from sub-cortical structures can be excluded because collaterals of geniculate afferents do not span sufficiently large distances and do not have oscillatory responses in this frequency range<sup>1,2</sup>.

We propose that the synchronization of oscillatory responses in spatially separate regions of the cortex may be used to establish a transient relationship between common but spatially distributed features of a pattern<sup>21</sup>. Our data show that synchronization is sensitive to global features of stimuli such as continuity, similarity of orientation and coherency of motion. Synchronization may therefore serve as a mechanism for the extraction and representation of global and coherent features

FIG. 1 Orientation-specific intercolumnar synchronization of oscillatory neuronal responses in area 17 of an adult cat. *a*, Normalized orientation tuning curves of the neuronal responses recorded from five electrodes spaced 400  $\mu$ m apart and centred on the representation of the area centralis. Response amplitudes (ordinate) to stimuli of different orientations (abscissa) are expressed as a percentage of the maximum response for each electrode. The arrows indicate the stimulus orientation ( $112^\circ$ ) at which the responses were recorded in *b*, *c* and *d*. *b*, Post-stimulus time histograms recorded simultaneously from the same five electrodes at an orientation of  $112^\circ$ . Note the small difference in the latencies of the responses indicating overlapping but slightly offset receptive field locations. *c*, Auto-correlograms of the responses recorded at sites 1 (1–1), 3 (3–3) and 5 (5–5). *d*, Cross-correlograms computed for the three possible combinations (1–3, 1–5, 3–5) between responses recorded on electrodes 1, 3 and 5. Correlograms computed for the first direction of stimulus movement are displayed with unfilled bars with the exception of comparison 1–5 in *d*.

**METHODS.** Adult cats were prepared for acute physiological recordings from the visual cortex using standard procedures<sup>2</sup>. Anaesthesia was induced with a short acting anaesthetic (hexobarbital, 15 mg per kg or ketamine, 15 mg per kg) and then supplemented with a mixture of 30%  $O_2$ , 70%  $N_2O$  and 0.1–0.3% halothane. Multi-unit activity was recorded from an array of 4–6 closely spaced (300–500  $\mu$ m) platinum-iridium electrodes (25  $\mu$ m tip diameter) and an additional single electrode that was moved independently. The array was inserted in the vicinity of the representation of the area centralis. The single electrode was positioned anteriorly and advanced down the medial bank of area 17. All receptive field locations were within  $15^\circ$  of the area centralis. Spikes exceeding a threshold of twice the noise level were detected with a window discriminator and digitized with a resolution of 1 ms. All recordings used binocular stimulation after the receptive fields for the two eyes had been aligned using prisms. When neurons recorded from different electrodes had overlapping receptive fields but differing orientation preferen-



ces, we used a stimulus orientation that evoked a response at each site of at least half the maximal amplitude. Responses that did not meet this criterion and that did not overlap in time were excluded from the analysis. In the case of non-overlapping receptive fields we applied two independently controllable stimuli. For each trial the stimuli were moved across the receptive fields, at the preferred velocity, in both directions of movement perpendicular to the axis of orientation. Each trial lasted for 10 s and was repeated 10 times.

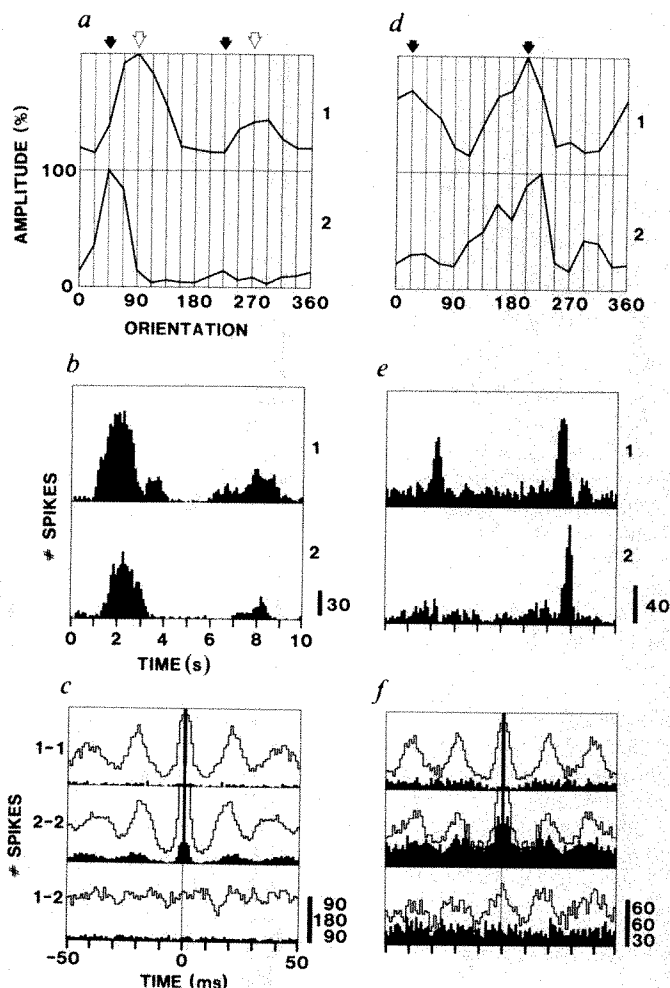


FIG. 2 Long-range inter-columnar synchronization of oscillatory responses depends on the similarity of orientation preference. *a*, Orientation tuning curves of multi-unit responses recorded at two sites (1 and 2) separated by 6 mm in area 17. The neurons had non-overlapping receptive fields and a clear difference in orientation preference of  $45^\circ$  (open and closed arrows). *b*, Post-stimulus time histograms of the responses recorded to stimulation of each receptive field at its own respective optimal orientation (open and closed arrows in *a*). *c*, Auto- (1-1, 2-2) and cross-correlograms (1-2) computed from the neuronal responses recorded from both electrodes revealed that oscillatory responses occurred at both sites but were not correlated. Unfilled bars are for the first direction of stimulus movement. *d*, Orientation tuning curves of multi-unit responses recorded from the same animal at two different cortical sites (1 and 2), separated by 7 mm. The neuronal responses at each site had similar orientation and directional preferences. *e*, Post-stimulus time-histograms of the activity recorded at each site in response to their optimal stimulus orientation of  $22^\circ$  (arrows in *d*). *f*, Auto- (1-1, 2-2) and cross-correlograms (1-2) computed from the neuronal responses recorded on the two electrodes demonstrate oscillatory responses at both sites which were correlated with zero phase difference. Unfilled bars are for the second direction of stimulus movement.

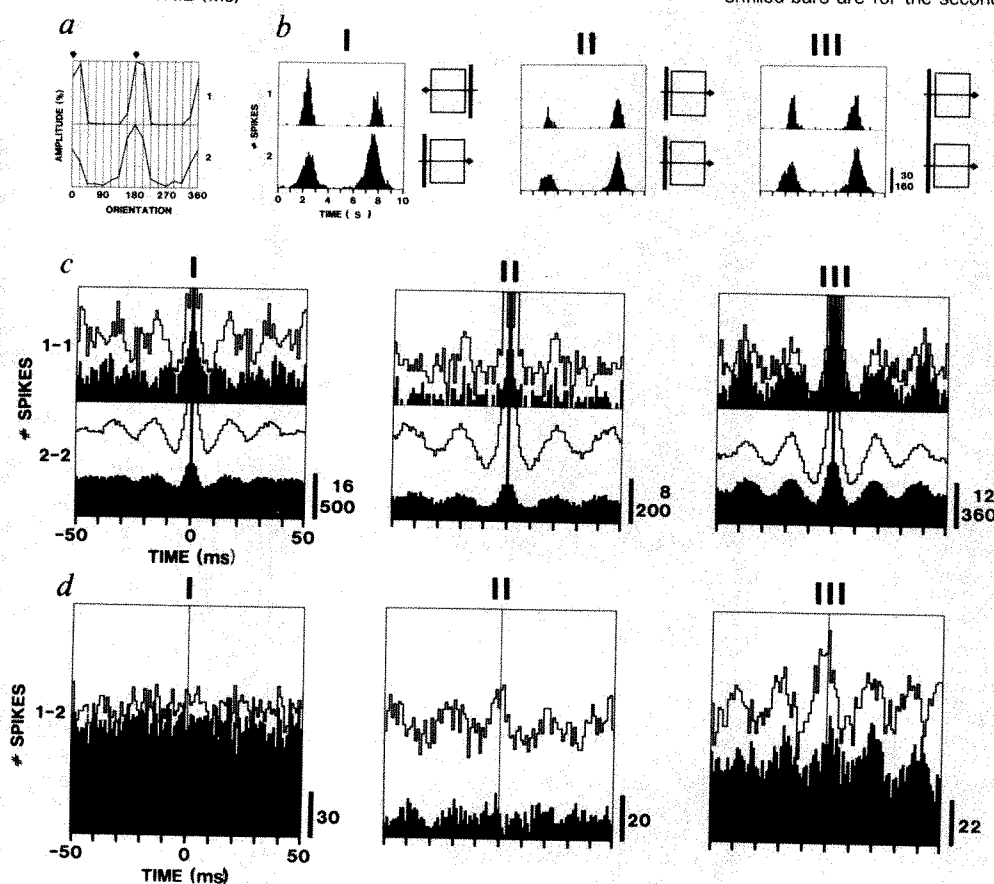


FIG. 3 Long-range oscillatory correlations reflect global stimulus properties. *a*, Orientation tuning curves of neuronal responses recorded from two electrodes (1, 2) separated by 7 mm show a preference for vertical light bars ( $0$  and  $180^\circ$ ) at both recording sites. *b*, Post-stimulus time-histograms of the neuronal responses recorded at each site for each of three different stimulus conditions: (I) two light bars moved in opposite directions; (II) two light bars moved in the same direction; and (III) one long light bar moved across both receptive fields. A schematic diagram of the receptive field locations and the stimulus configuration used is displayed to the right of each post-stimulus time histogram. *c*, *d*, Auto-correlograms (*c*, 1-1, 2-2) and cross-correlograms (*d*, 1-2) computed for the neuronal responses at both sites (1 and 2 in *a* and *b*) for each of the three stimulus conditions (I, II, III) displayed in *b*. For each pair of correlograms except the two displayed in *c* (I, 1-1) and *d* (I) the second direction of stimulus movement is shown with unfilled bars.

of a pattern. Such processes are crucial for the analysis of visual scenes and figure-ground segregation<sup>3,22-26</sup>. Synchronization of oscillatory responses may however also have a more general function in cortical processing because it is a powerful mechanism for establishing cell assemblies that are characterized by the phase and the frequency of their coherent oscillations. □

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## Transcripts of one of two *Drosophila* cyclin genes become localized in pole cells during embryogenesis

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CYCLINS, originally discovered in the eggs of marine invertebrates, are proteins which undergo dramatic cycles of synthesis followed by degradation at the metaphase-anaphase transition of cell division<sup>1-3</sup>. That they participate in the G<sub>2</sub>-M transition is supported by the fact that when synthetic cyclin messenger RNAs from clam and sea urchin are microinjected into the G<sub>2</sub>-arrested oocytes of *Xenopus*, they induce maturation<sup>2,4</sup>. The cyclin of fission yeast is the product of the *cdc13* gene, which is known to interact with *cdc2*, a gene required for the entry into mitosis<sup>5-10</sup>. We have cloned the genes that encode A-type and B-type cyclins from *Drosophila melanogaster* by virtue of their sequence similarity to oligonucleotides corresponding to conserved regions of the cyclin genes. We show that both genes encode abundant maternal mRNAs, but whereas the cyclin A mRNA is relatively uniformly distributed before cell formation, the cyclin B mRNA becomes localized to the developing pole cells. In larvae, cyclin A is expressed predominantly in brain and imaginal disks, whereas cyclin B transcripts are abundant in testes.

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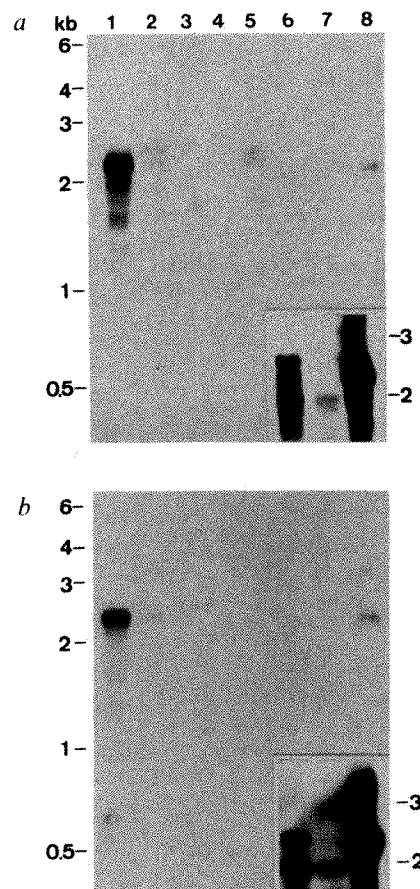


FIG. 1. Developmental northern blots showing the transcripts detected by cDNA clones of cyclins A (a) or B (b). Poly (A<sup>+</sup>) RNA from the following developmental stages was analysed: lane 1, 0-2 h embryos; lane 2, 2-4 h embryos; lane 3, first instar larvae; lane 4, second instar larvae; lane 5, third instar larvae; lane 6, pupae; lane 7, adult males; lane 8, adult females. The autoradiograph shown was exposed for 15 h. The inset in the lower right-hand corner of each autoradiograph is an 80-h (cyclin A) or 60-h (cyclin B) exposure in which only the region containing RNA of ~2-kb from lanes f-h is displayed. Filters were re-probed with the *Drosophila ras* gene (*Dmras64B*) (ref. 16) as a control for uniform loading of RNA. The autoradiograph exposures indicated that the cyclin transcripts are 1-2 orders of magnitude more abundant than *ras* RNA in *Drosophila* embryos.

**METHODS.** An adult female *Drosophila* cDNA library in the bacteriophage vector  $\lambda$ gt10 was screened with a <sup>32</sup>P-labelled oligonucleotide mixture of sequence: AA(A/G)TA(T/C)GA(A/G)GA(A/G)ATTA(T/C)CC (probe 1). Hybridizations and washings were carried out as recommended by Wood et al.<sup>17</sup>. Duplicate filters were screened in parallel with a second oligonucleotide mixture: AT(T/C/A)(C/T)T(G/A)TGA(T/C)-TGG(T/C)TGT (probe 2). Positive plaques were purified by rescreening following the same protocol. The cDNAs fell into two classes: those that hybridized to both probes, and those that hybridized only to probe 1, cyclins A and B, respectively. We isolated three cyclin A cDNAs and four cyclin B cDNAs from ~5 × 10<sup>7</sup> recombinant phage in this library. On rescreening, a 0-2 h embryo cDNA library, we isolated both cyclin A and B clones at a frequency of 0.002. The amino-acid sequence corresponding to probe 1 can be recognized within the amino-acid sequence derived from the sequence of the cDNA clones and is shown below in relation to amino acids 249-261 of clam cyclin A<sup>2</sup>, and the corresponding regions of sea urchin cyclin<sup>4</sup> and *cdc13* (refs 7 and 8):

Clam A	L	A	A	K	Y	E	E	I	Y	P	P	D	V
Sea urchin	I	A	S	K	Y	E	E	M	Y	P	P	E	I
<i>cdc13</i>	I	A	S	K	Y	E	E	V	M	C	P	S	V
<i>Drosophila</i> A	I	A	A	K	Y	E	E	I	Y	P	P	E	V
<i>Drosophila</i> B	I	A	T	K	Y	E	E	L	F	P	P	A	I

The amino-acid sequence corresponding to probe 2 (in relation to amino-acids 196-208 of clam cyclin A) is as follows:

Clam A	M	R	C	I	L	V	D	W	L	V	E	V	S
Sea urchin	M	R	L	I	L	V	D	W	L	V	Q	V	H
<i>cdc13</i>	M	R	G	I	L	T	D	W	L	I	E	V	H
<i>Drosophila</i> A	M	R	S	I	L	I	D	W	L	V	E	V	S
<i>Drosophila</i> B	M	R	A	V	L	I	D	W	I	N	E	V	H

The cyclin A cDNAs hybridize *in situ* to salivary gland chromosomes at 68E, and thus correspond to a gene that has been independently cloned by Lehner and O'Farrell<sup>18</sup> and Y.-N. Jan (personal communication). The cyclin B gene hybridizes *in situ* to chromosome 2 at 59A. Northern blots using the cloned cDNAs as probes have been described previously<sup>19</sup>.



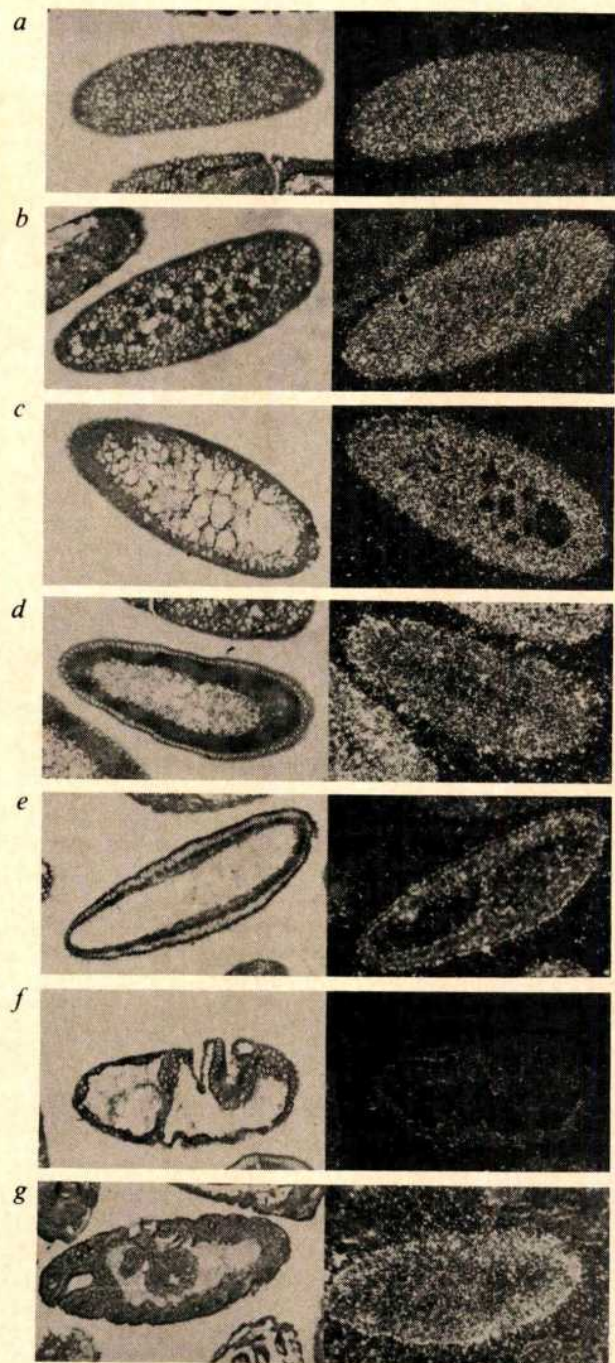


FIG. 2 *In situ* hybridization of a cyclin A cDNA probe to sections of embryos at various developmental stages. Bright-field and dark-field images are shown in the left and right panels respectively and embryos are oriented with the anterior pole to the left. Stages are according to Campos-Ortega and Hartenstein<sup>20</sup>. *a*, Early stage 2 embryo showing uniform distribution of signal. *b*, Late stage 2 embryo (nuclear cycle 7–8) showing uniform grain distribution. *c*, Early stage 4 embryo (nuclear cycle 10) in which the signal is becoming distributed toward the cortex. *d*, Late stage 4 embryo (division cycle 12). *e*, New cellular embryo (stage 6) with weaker signal throughout cellular region. *f*, Stage 8 embryo, mid-way through germ-band elongation showing extremely weak signal. *g*, Stage 11 embryo with fully elongated germ-band showing signal corresponding to zygotic transcripts. Hybridizations *in situ* were carried out according to refs 21 and 22, except that after labelling in a random oligonucleotide-priming reaction (20  $\mu$ l), the reactions were stopped by the addition of 1  $\mu$ l 0.5 M EDTA and 20  $\mu$ l 20 mM DTT. HCl (2  $\mu$ l, 5 M) was added and the incubation continued at 37 °C for 10 min, followed by the addition of NaOH (4  $\mu$ l, 5 M) and a further 20-min incubation. The reactions were neutralized with HCl (2  $\mu$ l, 5 M) and Tris-HCl (2  $\mu$ l, 2 M, pH 7.5).

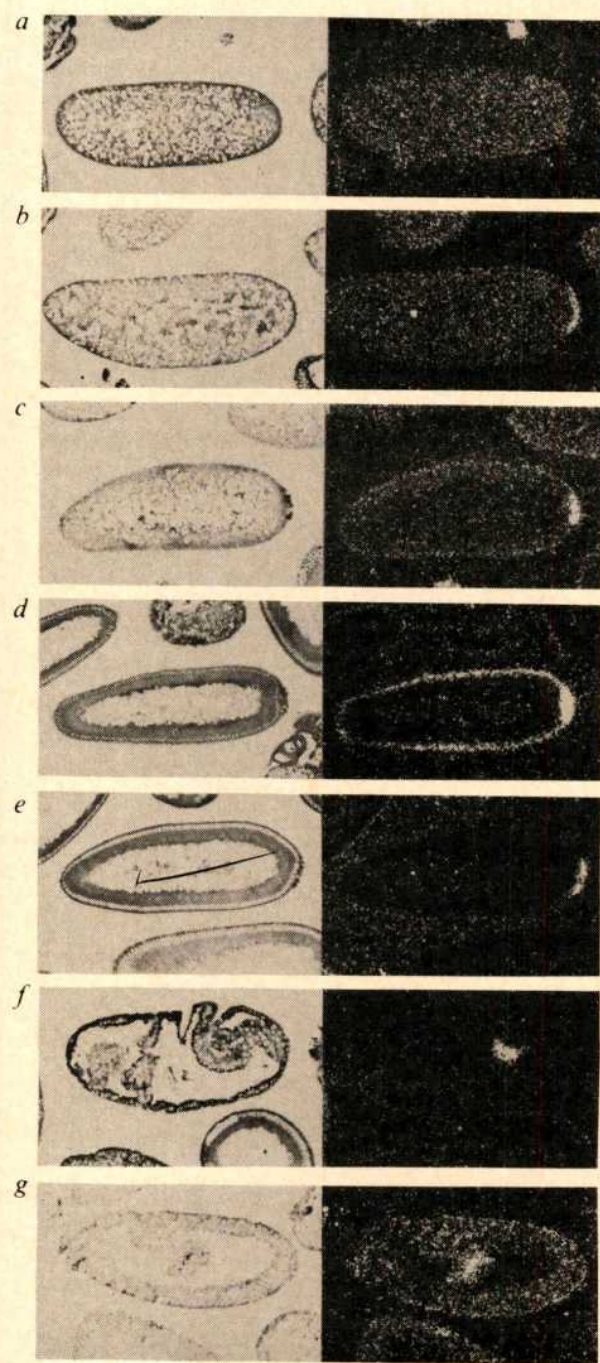


FIG. 3 *In situ* hybridization of a cyclin B cDNA probe to sections of embryos at various developmental stages. Bright-field and dark-field images are shown in the left and right panels, respectively. *a*, Early stage 2 embryo showing uniform distribution of signal. *b*, Late stage 2 embryo (nuclear cycle 8) showing transcripts beginning to concentrate at the posterior pole. *c*, Late stage 3 embryo (nuclear cycle 9) in which pole cells have just formed and are prominently labelled. Remaining labelling is becoming distributed towards the cortex. *d*, Late stage 4 embryo (division cycle 12) with all the signal tightly distributed in the vicinity of the nuclei at the cortex. Pole-cell labelling is conspicuous. *e*, An embryo forming cells (stage 6) in which somatic signal has disappeared leaving grains only over pole cells. *f*, Stage 8 embryo, mid-way through germ-band elongation showing labelling exclusively over pole cells. *g*, Stage 11 embryo with fully elongated germ-band showing signal prominent pole cell labelling and onset of signal corresponding to zygotic transcripts.



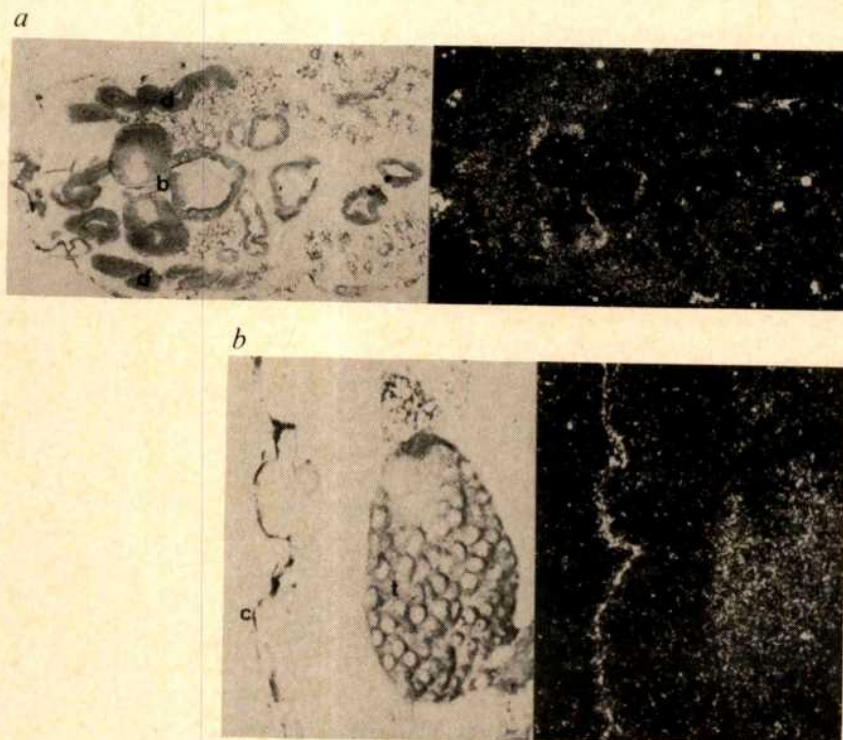


FIG. 4 *a*, *In situ* hybridization of a cyclin A cDNA probe to a section of a third instar larva. The anterior of the larva is facing to the left, and bright-field and dark-field images are shown in the left and right panels, respectively. The strongest labelling is seen in distinct regions of the three lobes of the brain (b). The imaginal disks (d) show a much lower level of more uniform labelling just above background. *b*, *In situ* hybridization of a cyclin B probe to a section of a third instar larva. This part of the section shows labelling of the testes (t) close to the larval cuticle (c). The signal in the cuticle is likely to be non-specific as it has been observed with many other probes (E.S., unpublished observations; M. Akam, personal communication). Fixation and embedding of larvae was carried out according to ref. 23.

We have cloned complementary DNAs corresponding to transcripts encoding proteins which, on the basis of sequence comparisons with the cyclins of other organisms, we refer to as cyclins A and B (see Fig. 1 legend). Both these cDNAs detect 2.3-kilobase (kb) transcripts that are most abundant in pre-cellular embryos, and also occur at high levels in adult females, as would be expected for maternally provided mRNA (Fig. 1). A 2.5-kb zygotic transcript of the cyclin A gene appears after cell formation (Fig. 1a, lane b), and is present at low levels throughout subsequent development. Three cyclin A transcripts are detectable in pupae, only one of which is present in adult males at low levels (Fig. 1a, inset). The developmental distribution of cyclin B transcripts is similar, except that a zygotic transcript of a different size is not seen, and a male-specific transcript of 2 kb is readily detectable (Fig. 1b inset, lane g).

The abundance of these transcripts in early embryos is consistent with the expected role of cyclins in the 13 cycles of mitosis that occur in the first 2 hours of development<sup>11,12</sup>. We have examined the distribution of transcripts during embryogenesis by *in situ* hybridization and find that maternal message is initially distributed uniformly throughout the embryo (Fig. 2a, b). There seems to be a broad redistribution of the cyclin A transcripts towards the cortex during cycles 8 and 9 which continues in subsequent cycles. This could be either an active process in which mRNA migrates with mitotic apparatus and nuclei, or a more passive process by which transcripts are excluded from the interior region of the embryo occupied by the coalescing yolk (Fig. 2c-e), although other explanations, including the differential degradation of RNA, are possible. The abundance of the cyclin A transcripts falls dramatically following cell formation (Fig. 2f). At later stages, the cell cycle lengthens to 1-2 hours and two or three additional rounds of division occur in spatially restricted domains of the embryo (ref. 13, and V. Foe, personal communication). During these stages, zygotic transcripts appear (Fig. 2g) which, we surmise, correspond to the 2.5-kb RNA seen in the northern blots (Fig. 1).

Maternal cyclin B transcripts are first found uniformly throughout the embryo (Fig. 3a). They then undergo a dramatic redistribution such that by cycles 8-9, when nuclei are migrating to the cortex, cyclin B transcripts are becoming concentrated at the posterior pole (Fig. 3b). It is also possible that this represents *de novo* transcription in the nuclei reaching the posterior pole.

These nuclei undergo cellularization several cycles before the majority to form pole cells (Fig. 3c). As the somatic nuclei complete their syncytial mitotic cycles, cyclin B transcripts show a clear association with the cortex near nuclei (Fig. 3d). These cortical transcripts disappear abruptly during cell formation of somatic nuclei (Fig. 3e). Transcripts either persist or continue to be produced in the pole cells as they move dorsally and anteriorly in germ-band elongation (Fig. 3f). Some zygotic cyclin B transcription seems to ensue in the soma in later embryos, although the predominant labelling is still detected in pole cells (Fig. 3g). The redistribution of cyclin B mRNA in the syncytial embryo suggests that these transcripts contain localization signals that allow them to remain close to dividing somatic nuclei and the developing pole cells. Other maternal mRNAs are known to have specific distributions in early embryos: the graded distribution of *bicoid* mRNA, with its maximum concentration at the anterior pole of the *Drosophila* embryo, for example, requires a specific sequence present at its 3' untranslated end<sup>14</sup>.

After the hatching of the embryo, most of ensuing larval development involves cell growth and polytenization in the absence of cell division. The exceptions are the imaginal tissues that must proliferate to form adult structures, neuroblasts within the larval brain, and the mitotic lineages of the germ cells. We find that expression of cyclin A, but not cyclin B, is readily detectable in the periphery of the three brain lobes, and, at a lower level, in imaginal disks of third instar larvae (Fig. 4a). Conversely, cyclin B transcripts predominate in the larval testes (Fig. 4b), and presumably correspond to the prominent male-specific 2-kb cyclin B RNA, detected by northern blotting (Fig. 1).

Several aspects of germ-cell proliferation are significantly different from mitotic cell division in somatic tissues. In the imaginal discs, for example, there is a target cell number that may be controlled by cell-cell contacts of a certain pattern<sup>15</sup>. By contrast, cell division continues in the stem cells of the testes and ovary throughout the life of the organism to produce daughter stem cells and primary gonial cells. In addition, the premeiotic mitoses of the gonial cells in both male and female are accompanied by incomplete cytokinesis to produce groups of cells that share cytoplasm connected by canals. In spermatogenesis, the gonial cells undergo 4 mitotic and 2 meiotic



divisions within cysts to produce 64 spermatids. In oogenesis a cyst of 16 interconnected cells develops, of which 14 will undergo polyploidization to form the nurse cells and one will become the oocyte, *per se*, and undergo meiosis. One might speculate that there is a need for a specific cyclin either to maintain stem-cell proliferation (to provide for the peculiarities of what are essentially syncytial divisions in the gonial cysts) or to mediate the ultimate transition between mitotic and meiotic divisions. □

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## A diacylglycerol analogue reduces neuronal calcium currents independently of protein kinase C activation

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DIACYLGLYCEROL analogues (for example 1,2-oleoylacetyl-glycerol, OAG) and phorbol esters are activators of protein kinase C, and have been widely used to study the function of this enzyme in both intact cells and cell-free preparations<sup>1,2</sup>. Electrophysiological studies have shown that these activators can either depress<sup>3–6</sup> or increase  $\text{Ca}^{2+}$  currents<sup>7–9</sup>, or decrease  $\text{K}^{+}$  currents<sup>10,11</sup> when applied outside the cell. It has been assumed that these effects are mediated by protein kinase C activation. Here we report that micromolar levels of OAG and phorbol esters depress  $\text{Ca}^{2+}$  currents in chick sensory neurons independently of their effect as activators of protein kinase C. The depression of the  $\text{Ca}^{2+}$  current is rapid and is unaffected by intracellular application of the protein kinase C inhibitors staurosporin, sphingosine and H-7<sup>12</sup>. Furthermore, the activators were ineffective when applied intracellularly, indicating that their site of action is on the outside of the membrane.

Intracellular patch-clamp recordings were performed in acutely isolated dorsal root ganglion cells from 10–13-day-old

chick embryos<sup>13,14</sup>. Figure 1a shows the change in action potential waveform induced by OAG<sup>15</sup> (5  $\mu\text{M}$ ), which was delivered by pressure application through a micropipette positioned 10–20  $\mu\text{m}$  from the cell. OAG reversibly depressed the amplitude of the spike and slightly prolonged its duration.

Under voltage-clamp, OAG reduced the total membrane current (see Fig. 1b), but isolated voltage-dependent  $\text{Na}^{+}$  currents, and also  $\text{K}^{+}$  currents<sup>3</sup>, were unaffected. A large, reversible decrease in the amplitude of isolated  $\text{Ca}^{2+}$  currents was recorded (Fig. 1c; see also ref. 3). A subsequent reduction of a  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  conductance could account for the decrease in the outward current shown in Fig. 1b.

$\text{Ca}^{2+}$  currents recorded under the conditions of Fig. 1c typi-

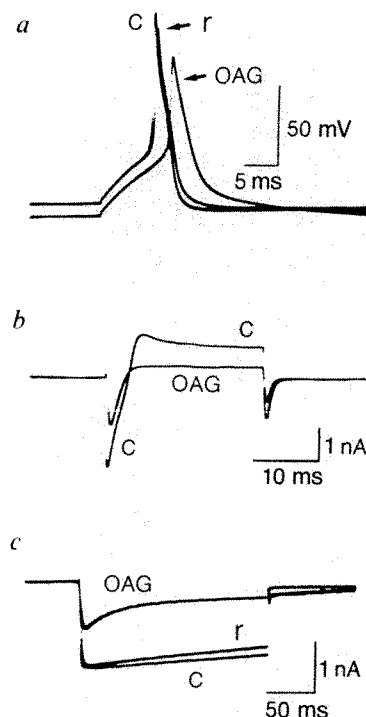


FIG. 1 Whole-cell recordings from chick dorsal root ganglion neurons before (c, control), during and after (r, recovery) 5-s exposures to 5  $\mu\text{M}$  OAG. a, OAG application reduced the action potential amplitude and delayed repolarization by several ms (resting potential  $-55$  mV). Recovery was 13 s after OAG application. Small hyperpolarizations (5–10 mV) were due to a non specific effect of the pressure application and were also observed when external saline was applied. b, Under voltage-clamp, both inward and outward currents were reversibly depressed by OAG (same cell as in a). c, OAG application reversibly reduced  $\text{Ca}^{2+}$  currents. Activating steps were from  $-50$  (b) and  $-80$  mV (c) to 0 mV.

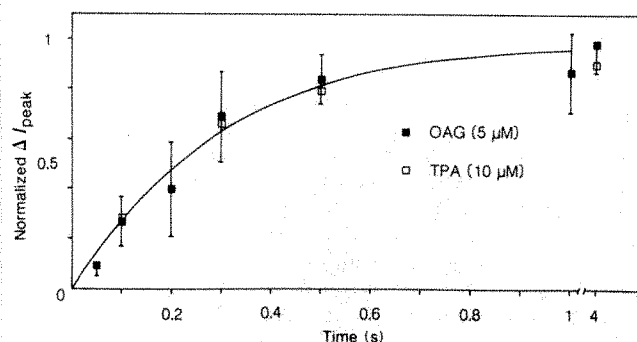
METHODS. Electrode resistances were 3–5 M $\Omega$ . Normal external salines contained (in mM): NaCl, 130; KCl, 5.4;  $\text{MgCl}_2$ , 2;  $\text{CaCl}_2$ , 2; HEPES 10 (pH 7.3). The internal electrode solution contained (in mM) potassium aspartate, 130; EGTA 10;  $\text{CaCl}_2$ , 1;  $\text{Mg-ATP}$ , 2; HEPES, 10 (pH 7.3).  $\text{Ca}^{2+}$  currents were recorded in isolation in external saline containing (in mM): choline chloride, 120;  $\text{CaCl}_2$ , 5;  $\text{MgCl}_2$ , 2; HEPES, 10 (pH 7.3) and internal solution containing (in mM) CsCl, 100; TEA-Cl, 20;  $\text{Mg-ATP}$ , 2; EGTA, 10;  $\text{CaCl}_2$ , 0.1; HEPES, 40 (pH 7.3). Sodium currents were analysed using external saline containing (in mM) NaCl, 120;  $\text{NiCl}_2$ , 5;  $\text{MgCl}_2$ , 2; HEPES, 10 (pH 7.3). Potassium currents were analysed using external saline containing (in mM) choline chloride, 150;  $\text{NiCl}_2$ , 5;  $\text{MgCl}_2$ , 2; HEPES, 10 (pH 7.3) and internal electrode solution containing (in mM) potassium aspartate, 130;  $\text{MgCl}_2$ , 2; EGTA, 10; HEPES, 10 (pH 7.3). The temperature was 22  $^{\circ}\text{C}$ . Stock solutions of OAG (50 mM, Sigma) were prepared in chloroform and diluted in external saline. OAG did not immediately dissolve in the chloroform, as solutions tested the same day had no effect. Stock solutions were then stored for 3–4 weeks at 4  $^{\circ}\text{C}$  after which they did depress  $\text{Ca}^{2+}$  currents. Stock solutions of  $\text{dCa}_8$  (50 mM, Sigma), sphingosine (200 mM, Sigma) and H-7 (50 mM, Sigma) were prepared in chloroform and stock solutions of staurosporin (1 mM, Boehringer) were prepared in dimethylsulphoxide. Phorbol esters (1–11 mM, Sigma) were dissolved in ethanol. Stock solutions were stored at 4  $^{\circ}\text{C}$ . Sonification improved the solubility of the compounds.

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FIG. 2 Rate of reduction of the  $\text{Ca}^{2+}$  current during application of 5  $\mu\text{M}$  OAG ( $n=5$ ) and 10  $\mu\text{M}$  TPA ( $n=2$ ). The measurements were made using a delivery system that exchanges the solution surrounding the cell in about 50 ms (ref. 22). The difference between peak currents recorded after prolonged washout and during drug applications of various duration was normalized to the difference between peak currents recorded after washout and after prolonged (20 s) drug application.



cally inactivated with a time constant of several hundred ms, as described for high-threshold (L-type)  $\text{Ca}^{2+}$  currents<sup>16-19</sup>. During OAG application, the reduced  $\text{Ca}^{2+}$  current inactivated faster when 0.1% chloroform was used as a solvent but not with lower concentrations. Recovery was slower than that of the peak current. Similar results were obtained with another diacylglycerol analogue, *sn*-1,2-dioctanoylglycerol ( $\text{diC}_8$ )<sup>20</sup>, and the three phorbol esters, *O*-tetradecanoylphorbol-13-acetate (TPA, phorbol myristyl acetate), phorbol-12,13-diacetate (PDA) and 4- $\alpha$ -phorbol-12,13-didecanoate (4- $\alpha$ -PDD).

We did not record shifts in the current-voltage relationship or changes in the cell leakage conductance during application of these agents (see also refs 3-5). Steps from -80 mV to 0 mV activated both high and low threshold (T-type)  $\text{Ca}^{2+}$  currents<sup>16-19</sup>. We found that the T-type current, which constitutes about 15% of the total  $\text{Ca}^{2+}$  current, was also transiently and reversibly blocked by OAG; similar observations have been made in GH3 cells<sup>5</sup>.

Table 1 (top) summarizes our results with diacylglycerol analogues and phorbol esters. Phorbol esters and  $\text{diC}_8$  were less effective than OAG even at 10-fold higher concentrations. Control solutions of either glycerol or the solvents had no significant effect on the peak  $\text{Ca}^{2+}$  current. Interestingly, 4- $\alpha$ -PDD, which does not activate protein kinase C<sup>21</sup>, was as effective as the other phorbol esters at depressing  $\text{Ca}^{2+}$  currents.

TABLE 1 Effects of activators and inhibitors of protein kinase C

External pipette	Internal pipette	Per cent decrease $I_{\text{peak}}$ (mean $\pm$ s.d.)	<i>n</i>
0.5 $\mu\text{M}$ OAG	—	2 $\pm$ 2	7
5 $\mu\text{M}$ OAG	—	58 $\pm$ 24	18
5 $\mu\text{M}$ $\text{diC}_8$	—	2 $\pm$ 1	3
50 $\mu\text{M}$ $\text{diC}_8$	—	18 $\pm$ 9	6
50 $\mu\text{M}$ glycerol	—	0 $\pm$ 2	3
10 $\mu\text{M}$ TPA	—	18 $\pm$ 5	6
10 $\mu\text{M}$ PDA	—	15 $\pm$ 5	6
10 $\mu\text{M}$ 4- $\alpha$ -PDD	—	17 $\pm$ 3	3
0.1% chloroform	—	3 $\pm$ 1	4
0.1% ethanol	—	3 $\pm$ 2	4
5 $\mu\text{M}$ OAG	1 $\mu\text{M}$ staurosporin	51 $\pm$ 17	6
5 $\mu\text{M}$ OAG	50 $\mu\text{M}$ sphingosine	75 $\pm$ 15	6
5 $\mu\text{M}$ OAG	200 $\mu\text{M}$ sphingosine	89 $\pm$ 9	5
5 $\mu\text{M}$ OAG	50 $\mu\text{M}$ H-7	72 $\pm$ 12	5
10 $\mu\text{M}$ TPA	50 $\mu\text{M}$ H-7	12 $\pm$ 6	7
10 $\mu\text{M}$ PDA	50 $\mu\text{M}$ H-7	8 $\pm$ 5	5
50 $\mu\text{M}$ $\text{diC}_8$	50 $\mu\text{M}$ H-7	30 $\pm$ 10	6
1 $\mu\text{M}$ staurosporin	—	2 $\pm$ 1	3
50 $\mu\text{M}$ sphingosine	—	5 $\pm$ 4	3
200 $\mu\text{M}$ sphingosine	—	32 $\pm$ 8	3
50 $\mu\text{M}$ H-7	—	31 $\pm$ 10	5

All agents were delivered through micropipettes for either 5 s externally or for a minimum of 10 min internally. Chloroform was the solvent for OAG and  $\text{diC}_8$ , and ethanol was used to dissolve the phorbol esters (see text for details).  $I_{\text{peak}}$ , peak voltage-dependent  $\text{Ca}^{2+}$  current; *n*, number of cells examined.

We measured the onset of  $\text{Ca}^{2+}$  current reduction by OAG and TPA using a rapid delivery system<sup>22</sup> (Fig. 2). Inhibition developed in less than 50 ms and was half-maximal by 200 ms. The offset of the response was similarly fast, indicating that the action was probably limited by diffusion. These results indicate that the drugs may be acting on the outside of the membrane, and not through an enzymatic pathway on the inner membrane.

To examine this possibility more directly, we tested whether the activators could depress  $\text{Ca}^{2+}$  currents after inhibition of protein kinase C. Table 1 (middle) summarizes our results from cells perfused internally with either staurosporin<sup>23</sup> sphingosine<sup>24,25</sup> or H-7<sup>26</sup>. We used H-7 and staurosporin at concentrations that were 10 and 100 times their respective inhibition constants ( $K_i$ ) (obtained from *in vitro* studies<sup>23,25</sup>). As the  $K_i$  for sphingosine is unknown, we used concentrations of 50  $\mu\text{M}$  and 200  $\mu\text{M}$ , the latter reducing protein kinase C activity by 90% in several cell types<sup>24,25</sup>. The inhibitors had no significant effect on the OAG responses. All values were obtained a minimum of 10 min after the start of cell perfusion and, in several cells, after 30 min. With 2 mM ATP and 40 mM HEPES buffer in the internal pipette, there was no evidence for rundown of the  $\text{Ca}^{2+}$  current before 30 min (see also refs 27 and 28). Similarly, internal H-7 (50  $\mu\text{M}$ ) had no effect on the  $\text{diC}_8$  or phorbol ester responses (Table 1, centre). Sphingosine and H-7, applied at the same concentration to the outside of cells, quickly and reversibly reduced the  $\text{Ca}^{2+}$  current (Table 1, bottom).

In a different approach, we tested whether OAG was effective if applied intracellularly. For these experiments, we used two

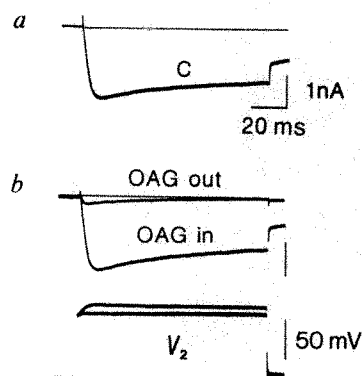


FIG. 3 Current and voltage measurements using a three-electrode recording arrangement to compare the effects of OAG applied inside (50  $\mu\text{M}$ ) and outside (5  $\mu\text{M}$ ) to the same cell. *a*, Control record (*c*) of the  $\text{Ca}^{2+}$  current recorded with the first patch electrode during a voltage step from -80 mV to 0 mV. *b*, 10 min after the whole-cell configuration was established with the second (OAG) patch electrode, the  $\text{Ca}^{2+}$  current was unchanged (OAG in). External application of OAG through a third microelectrode was still effective at blocking the  $\text{Ca}^{2+}$  current (OAG out) however. Membrane voltage with the second patch electrode ( $V_2$ ) was recorded in the current-clamp mode. The slowly decaying tail currents in the control and internal OAG records may indicate inadequate clamping on repolarization as they were not present under better recording conditions<sup>13</sup>.

patch pipettes, one filled with control internal saline and the other with internal saline plus 50  $\mu$ M OAG (Fig. 3). This high concentration was necessary because OAG is rapidly metabolized<sup>15</sup>. In all seven experiments we found no change in the  $\text{Ca}^{2+}$  current during intracellular OAG application even after 25 min (average duration was 15 min). In five cases, we applied OAG externally from a third electrode and recorded the usual OAG decrease of the current (Fig. 3b). In three similar experiments using H-7 (50  $\mu$ M) in the second electrode instead of OAG, there was no measurable effect on the  $\text{Ca}^{2+}$  current. Because micromolar  $\text{Ca}^{2+}$  concentrations are necessary for protein kinase C activity in the absence of diacylglycerol analogues<sup>1</sup>, total  $\text{CaCl}_2$  concentration in the internal solution was raised for these experiments from 1 mM to 9.1 mM to increase the free  $\text{Ca}^{2+}$  concentration from 0.1  $\mu$ M to 1  $\mu$ M.

We conclude that OAG and H-7 act on the outside of dorsal root ganglion neurons to depress the  $\text{Ca}^{2+}$  current. We suspect that the other agents tested act in the same way, although our evidence is less direct. At the inhibitor concentrations used, both protein kinase C and cyclic AMP-dependent and cyclic GMP-dependent protein kinases should have been completely inhibited, indicating that the functioning of  $\text{Ca}^{2+}$  channels in dorsal root ganglion neurons does not depend on these protein kinases. This might not be the case in all cell types<sup>29-31</sup>.

The demonstration of external action of activators and inhibitors of protein kinase C is reminiscent of recent results for forskolin, an adenyl cyclase activator. Several studies have presented evidence that forskolin may affect  $\text{Ca}^{2+}$  currents<sup>32</sup> and  $\text{K}^{+}$  currents<sup>33-36</sup> independently of cyclase activity. Like forskolin, OAG and H-7 did not affect the  $\text{Ca}^{2+}$  current when applied intracellularly. These molecules apparently possess little affinity for the  $\text{Ca}^{2+}$  channel on the inner side of the membrane, although the fast on-kinetics suggest a high-affinity external binding site. Direct antagonistic effects on  $\text{Ca}^{2+}$  channels must be considered when applying these agents extracellularly to study events involving protein kinase C. □

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## Linkage of a prion protein missense variant to Gerstmann-Sträussler syndrome

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GERSTMANN-Sträussler syndrome is a rare familial neurodegenerative condition that is vertically transmitted, in an apparently autosomal dominant way<sup>1</sup>. It can also be horizontally transmitted to non-human primates and rodents through intracerebral inoculation of brain homogenates from patients with the disease<sup>2-8</sup>. The exact incidence of the syndrome is unknown but is estimated to be between one and ten per hundred million. Patients initially suffer from ataxia or dementia and deteriorate until they die, in one to ten years. Protease-resistant prion protein (PrP) and PrP-immunoreactive amyloid plaques with characteristic morphology accumulate in the brains of these patients<sup>9-11</sup>. Current diagnostic criteria for Gerstmann-Sträussler syndrome incorporate clinical and neuropathological features, as animal transmission studies can be unreliable<sup>8,12</sup>. PrP is implicated in the pathogenesis and transmission of the condition and in scrapie, an equivalent animal disease<sup>13-17</sup>. It was discovered by enriching scrapie-infected hamster brain fractions for infectivity<sup>18,19</sup>. Because there is compelling evidence that the scrapie isoform of PrP is a necessary component of the infectious particle<sup>15,16</sup>, it seemed possible that the PrP gene on the short arm of human chromosome 20 (ref. 20) in Gerstmann-Sträussler syndrome might be abnormal. We show

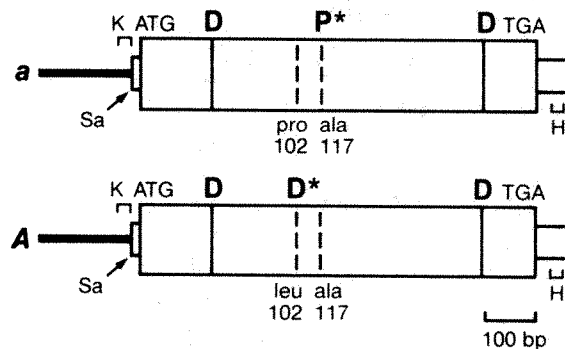


FIG. 1 Organization of PrP alleles in ataxic GSS. Top, Wild-type PrP allele (a); bottom, variant PrP allele found in ataxic GSS (A). The open reading frame is indicated by an open box, the mRNA untranslated region by a narrower box and the intron by a solid black line. Restriction sites: P, *PvuII*; D, *DdeI*. Sa indicates a putative 3' splice site (splice acceptor). Asterisks indicate locations of polymorphic restriction sites; broken vertical lines indicate locations of polymorphic codons. Brackets indicate locations of synthetic primers K (5'-AAGAATTCTCTGACATTCCTCTTCA-3') and H (5'-AAGGATCCCTCAA-GCTGGAAAAAG-3') used in cyclic thermal DNA amplifications. The K oligonucleotide is based on unpublished sequence data of the intron in the human PrP gene; a partial sequence of the intron 5' to the open reading frame of the A allele and including the initiation codon is: 5'-CATTATGCAGAAACATTT-AGTAAATCAACATAAATATGGAACCTCTGACATTCCTCTTCTTTCAGAGCA-GTCATTATG-3'. This data was obtained during sequencing of the two PrP alleles in patient JJ. H and K oligonucleotides were constructed to include 5'-*Bam*HI and *Eco*RI linkers, respectively.

here that PrP codon 102 is linked to the putative gene for the syndrome in two pedigrees, providing the best evidence to date that this familial condition is inherited despite also being infectious, and that substitution of leucine for proline at PrP codon 102 may lead to the development of Gerstmann-Sträussler syndrome.

We sequenced both alleles of the PrP open reading frame in J.J., a patient with Gerstmann-Sträussler syndrome (GSS) from a US pedigree (see III-a, Fig. 2a). Five recombinant bacteriophage containing the PrP open reading frame were recovered from a  $\lambda$ -EMBL-3 library constructed from the patient's peripheral leukocyte DNA and screened with a human PrP cDNA<sup>21</sup>. There are two PrP alleles, which can easily be distinguished in genomic DNA and recombinant clones because one allele differs from the other by the absence of a *PvuII* restriction site located in the PrP open reading frame. This polymorphism has been reported previously; the *PvuII* site is present in 90% of the population<sup>22</sup>. We found that the *PvuII* site was abolished by a 'silent' adenine to guanine substitution in the third position of alanine codon 117. One of the five clones lacked this *PvuII* site. In the same clone, a cytosine to thymine substitution in the second position of codon 102 resulted in a proline to leucine change, creating a *DdeI* restriction site (Fig. 1). No other variations between the two alleles were found. The other allele was identical to another published PrP gene sequence<sup>21</sup> and is presumably the wild-type allele.

To determine whether the leucine substitution at codon 102 was present in other individuals we used either Southern blotting analysis with *DdeI*-digested genomic DNA, or restriction digests of genomic DNA amplified by the polymerase chain reaction (Fig. 1) visualized directly with ethidium bromide (see Fig. 3). We found the leucine substitution in the two other individuals (J.B. and J.C.) that we examined with ataxic GSS, both of whom are members of an extensive British pedigree (see III-20 and

IV-2, Fig. 2, lower pedigree). The leucine substitution was not present in eight unaffected relatives of J.J., J.B., or J.C. who are beyond the age for being at risk for the disease (Figs 2 and 3). Nor was it found in 15 patients with other forms of inherited or sporadic prion diseases, or in 100 unrelated, normal Caucasian individuals of the same ethnic background as the British and US pedigrees.

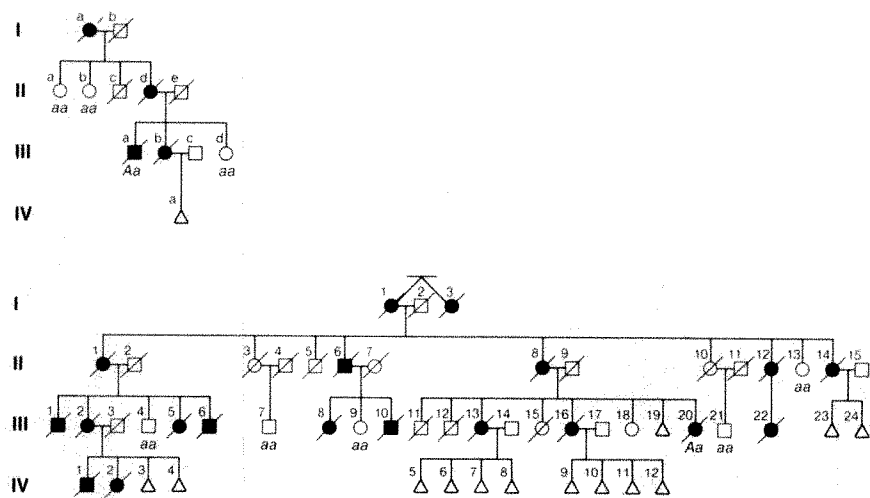
The family data were analysed for genetic linkage using the LIPED computer program<sup>23</sup>. We assumed that the disease followed a dominant mode of inheritance with age-dependant penetrance, where penetrance was assumed to rise linearly from 0 at age 21 to 100% at age 40 in the US family and from 0 at age 38 to 100% at age 66 in the British family. Under these conditions, the log likelihood ratio (lod score) is lowered by asymptomatic individuals with the leucine substitution who are older than the minimum age of onset. As the disease is known to be extremely rare, a population frequency of  $q=1$  in 10,000,000 was assumed for the disease gene, corresponding to a disease prevalence of 2 in 10,000,000 among older individuals beyond the age at risk. Even for values of  $q$  as high as 0.0001, the results in Table 1 are unaltered.

The population frequency of the leucine substitution,  $p$ , is crucial for the linkage analysis as many married-in spouses are of unknown genotype at this marker locus. When the substitution is rare, it is probably inherited from one of the founding members in each pedigree who also passed the disease gene to his or her offspring. None of a random sample of 100 Caucasian individuals carried the substitution (no substitution in 200 alleles) so that the 95% confidence interval for the allele frequency,  $p$ , extends from 0 to  $1 - 0.05^{0.005} = 0.015$  and the 99% confidence interval for  $p$  ranges from 0 to 0.023.

The results shown in Table 1 demonstrate significant tight linkage between the disease and the marker locus, the maximum

FIG. 2 Pedigrees of two families with ataxic GSS. Circles, females; squares, males; triangles, males or females; a black symbol indicates that an individual is affected with GSS; a slashed symbol that an individual is deceased. The clinical and pathological aspects of these pedigrees will be described in detail elsewhere. On the basis of the predicted amino-acid sequence, *a* indicates a proline at codon 102 and *A* indicates a leucine at that codon. Because of their predictive nature, the alleles for individuals at risk for illness (triangles) are not shown in order to protect the confidentiality of these people. The distribution of the candidate alleles in the at-risk group was found to be in accordance with that expected for an autosomal dominant trait. Detailed information of allelic assignments on which the lod score calculations are based will be made available to investigators for scientific purposes only but in strict confidence.

Top, Pedigree of US family with ataxic GSS. This is a previously unreported pedigree. IV-a (age 23) developed leg pains and gait instability in 1988, but is scored as an individual at risk for illness in the absence of a more definitive diagnosis. The diagnosis of GSS was confirmed in III-a and III-b by positive staining of amyloid plaques with anti-PrP antibodies. The known ages (in years) of onset for affected individuals are: I-a, 40; II-d, 22; III-a, 39; and III-b, 29. Hamsters which received intracerebral inoculations of a 10% brain homogenate from III-a are still asymptomatic after 500 days. Both alleles of the PrP open reading frame were sequenced in the index case, III-a, using a combination of methods described by Maxam and Gilbert<sup>35</sup> and Biggin and co-workers<sup>36</sup>. Bottom, Pedigree of a British family with GSS. This pedigree has been extensively analysed, both clinically and pathologically<sup>4,6,37,38</sup>. PrP-positive plaques were demonstrated in III-20 and IV-2. The known ages (in years) of onset for affected individuals are: II-6, 52; II-8, 46; II-12, 66; III-1, 65; III-2, 54; III-5, 57; III-6, 60; III-8, 48; III-10, 42; III-13, 51; III-16, 39; III-20, 48; III-22, 59; and IV-2, 40. The ages of individuals at risk for illness are: III-19, 58; III-23, 58; III-24, 51; IV-3, 53; IV-4, 51; IV-5, 38; IV-6, 34; IV-7, 32; IV-8, 30; IV-9, 40; IV-10, 39; IV-11, 35; and IV-12, 34. Hamsters which received intracerebral inoculations of 10% brain homogenates from III-20 and IV-2



developed neurological disease at  $317 \pm 21.8$  ( $n=5$ ) and  $300 \pm 11.2$  ( $n=8$ ) days, respectively, and died at  $327 \pm 19.6$  and  $305 \pm 11.5$  days, respectively. Transmission of brain homogenates from IV-2 (ref. 6) and III-10 (ref. 4) to monkeys has been reported previously.

**METHODS.** Relevant family members were examined by a neurologist and blood samples were obtained. DNA was extracted from the buffy coat in all instances except IV-2 for which only frozen brain was available. DNA from III-20 and IV-2 was extracted from freshly frozen tissue. We also extracted DNA using a previously published method<sup>34</sup> from formalin-fixed brains from several other affected members of these two pedigrees, for example III-b, III-10 and III-22, and from a single patient from a third pedigree. We found discrepancies between data obtained from matched control samples of DNA from fresh and formalin-fixed tissue, however. Therefore, even though the mutant *DdeI* site was present in each of these four additional GSS cases, we excluded these data from our analysis. Codon 102 of the PrP open reading frame was determined by digestion with *DdeI* of DNA amplified between the primers described in Fig. 1.



lod score being equal to  $Z = 3.26$  at a recombination fraction of  $\Theta = 0$  ( $p = 0.01$ ). The commonly used approximate confidence interval for  $\Theta$  extends from 0 to 0.14, which is the value of  $\Theta$  with a lod score of  $Z = 1$ .

The US and British pedigrees seem to be lineally distinct both on the basis of currently available genealogical inquiries and comparison of the restriction map in the PrP open reading frame. The *PvuII* polymorphism at codon 117 is present in the US family but not in the British family. Although more extensive genealogical investigation could conceivably reveal common ancestry between these two pedigrees, at present it seems more likely that the mutation causing the leucine substitutions at codon 102 arose independently in the two pedigrees. This base substitution may have involved deamination of a methylated cytosine situated 5' to guanine and therefore represents a relatively common mutation in human DNA<sup>24</sup>. The proline at codon 102 seems to be highly conserved as all rodent PrP genes sequenced so far also encode a proline at the equivalent codon<sup>14,24-29</sup>.

The codon 102 proline→leucine substitution is possibly located within a surface loop which is nine residues from a putative transmembrane  $\alpha$ -helix<sup>30</sup>. It would therefore be unlikely to exert a significant effect on the secondary structure of PrP. The biological consequences of single amino-acid substitutions that do not perturb secondary structure can nevertheless be profound, as in certain haemoglobinopathies and neoplasias<sup>31-33</sup>.

We believe that, on the basis of clinical and pathological criteria, GSS can be classified into three forms: an ataxic form, for which the leucine substitution seems to be characteristic; a dementing form; and a dementing form that is accompanied by pathological quantities of neurofibrillary tangles. When familial Creutzfeldt-Jakob disease is included, at least four forms of human inherited prion diseases can be deciphered<sup>40</sup>. A fuller

TABLE 1 Lod scores for the two families for several values of  $p$ , the leucine substitution frequency

$p$	Recombination fraction				
	0	0.05	0.10	0.15	0.20
0.023	0.634	0.521	0.411	0.308	0.215
	<u>2.471</u>	<u>2.238</u>	<u>1.989</u>	<u>1.723</u>	<u>1.441</u>
	3.105	2.759	2.400	2.031	1.656
0.015	0.640	0.526	0.415	0.311	0.218
	<u>2.562</u>	<u>2.328</u>	<u>2.077</u>	<u>1.809</u>	<u>1.524</u>
	3.202	2.854	2.492	2.120	1.742
0.01	0.643	0.529	0.418	0.313	0.219
	<u>2.617</u>	<u>2.382</u>	<u>2.130</u>	<u>1.860</u>	<u>1.573</u>
	3.260	2.911	2.548	2.173	1.792
0.001	0.650	0.534	0.422	0.317	0.222
	<u>2.707</u>	<u>2.471</u>	<u>2.218</u>	<u>1.946</u>	<u>1.655</u>
	3.357	3.005	2.640	2.263	1.877
0.0001	0.650	0.535	0.423	0.317	0.222
	<u>2.715</u>	<u>2.479</u>	<u>2.225</u>	<u>1.953</u>	<u>1.663</u>
	3.365	3.014	2.648	2.270	1.885
$10^{-7}$	0.650	0.535	0.423	0.317	0.222
	<u>2.717</u>	<u>2.480</u>	<u>2.227</u>	<u>1.954</u>	<u>1.664</u>
	3.367	3.015	2.650	2.271	1.886

The format is: lod scores for the US family + lod scores for the British family = summed lod scores for both families.

description of this proposed classification will be published elsewhere.

The linkage between the PrP gene and GSS in two pedigrees establishes, for the first time, that GSS is a genetic disorder. Our observations parallel earlier studies where the murine PrP gene was found to be linked to susceptibility (that is, length of the incubation time) for experimental scrapie after inoculation with prions<sup>13</sup>. Moreover, our discovery of a unique PrP gene amino-acid substitution on the same chromosome that carries the GSS locus in two pedigrees also resembles earlier studies in which substitutions of two amino-acid residues at PrP codons 108 and 189 correlate with short and long incubation times in experimental scrapie studies in mice<sup>14</sup>. If the leucine substitution at codon 102 arose independently in our two pedigrees, then we believe it could represent more than a linked genetic marker and might itself provoke the development of GSS. Although the composition of the GSS prion has not been determined directly, it seems likely, by analogy with results from experimental scrapie studies in rodents, that the GSS prion is composed largely, if not entirely, of an abnormal isoform of human PrP. The striking and specific correlation between the leucine substitution and the ataxic form of GSS leads us to speculate that other specific mutations in PrP may correlate with the other forms of inherited prion diseases. □



FIG. 3 Restriction patterns of twelve informative cases from the families with ataxic GSS shown in Fig. 2.

**METHODS.** Genomic DNA extracted from peripheral leukocytes was amplified using the *Thermus aquaticus* (Taq) heat-stable DNA polymerase as previously described<sup>39</sup>. Amplification reactions used 1–2  $\mu$ g of DNA in a 100  $\mu$ l solution containing 1  $\mu$ M each of primer KH10 and KH11 and 2.5 units Taq polymerase in the reaction buffer provided by the supplier (Perkin-Elmer-Cetus). The samples were overlaid with two drops of mineral oil to prevent evaporation and were subjected to 35 cycles of amplification. The cycling reaction was performed in a programmable heat block (DNA Thermal Cycler, PECL) set to incubate the samples at 94 °C for 90 s (to denature the DNA), at 55 °C for 60 s (to anneal the primers), and at 72 °C for 3 min (to extend the annealed primers). The reaction product (30–60  $\mu$ l) was fractionated on a preparative 1.5% agarose gel from which an 864-base pair fragment was excised and purified using GeneClean (Bio 101). The excised fragment (30 mg) was digested to completion with 5 units *DdeI* in a volume of 12  $\mu$ l using the buffer recommended by the supplier. The DNA was fractionated on a 3%:1% NuSieve-SeaKem agarose gel (FMC Corp.) containing ethidium bromide (0.5  $\mu$ g ml<sup>-1</sup>) in 40 mM Tris-acetate, 2 mM EDTA buffer. *HaeIII*-digested  $\phi$ X 174 DNA (250 ng) was loaded as a size marker.

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## Polymorphism in the $\alpha_3$ domain of HLA-A molecules affects binding to CD8

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**CYTOTOXIC T lymphocytes (CTL) expressing the CD8 glycoprotein recognize peptide antigens presented by class I major histocompatibility complex (MHC) molecules<sup>1,2</sup>. This correlation and the absence of CD8 polymorphism led to the hypothesis that CD8 binds to a conserved site of class I MHC molecules. Using a cell–cell binding assay we previously demonstrated specific interaction between human class I MHC (HLA-A,B,C) molecules and CD8 (ref. 3). Subsequent analysis of the products of 17 HLA-A,B alleles revealed a natural polymorphism for CD8 binding in the human population. Two molecules, HLA-Aw68.1 and HLA-Aw68.2, which do not bind CD8, have a valine residue at position 245 whereas all other HLA-A,B,C molecules have alanine. Site-directed mutagenesis shows that this single substitution in the  $\alpha_3$  domain is responsible for the CD8 binding phenotype and also affects recognition by alloreactive and influenza-specific CTL. Our results indicate that CD8 binds to the  $\alpha_3$  domain of class I MHC molecules.**

Various class I MHC alleles were transfected into the HLA-A,B negative B-cell line, CIR. The resulting transfectants were tested for binding to Chinese hamster ovary (CHO) cells expressing the human CD8 gene, using the assay described by Norment *et al.*<sup>3</sup>. Fifteen of 17 HLA-A,B molecules tested gave significant levels of positive binding, as did two murine class I MHC

TABLE 1 Binding of CD8 to class I molecules

Experiment	Transfected class I gene	Cells bound per well ( $\times 10^{-3}$ )	
		CD8 <sup>+</sup>	CD8 <sup>-</sup>
I	HLA-A2.1	32.2 $\pm$ 6.4	8.8 $\pm$ 0.8
	A2.2Y	38.8 $\pm$ 1.0	11.2 $\pm$ 1.0
	A2.3	33.1 $\pm$ 1.9	9.5 $\pm$ 1.2
	Aw69	25.6 $\pm$ 3.0	12.5 $\pm$ 0.9
	A3.1	49.6 $\pm$ 1.2	9.2 $\pm$ 1.8
	A3.2	31.4 $\pm$ 0.6	5.3 $\pm$ 0.4
	A1	27.2 $\pm$ 1.4	9.7 $\pm$ 2.1
	A24	28.3 $\pm$ 1.6	6.5 $\pm$ 1.2
	Aw68.1	15.1 $\pm$ 1.8	8.2 $\pm$ 1.3
	Aw68.2	13.9 $\pm$ 1.0	9.7 $\pm$ 1.3
II	untransfected CIR	18.2 $\pm$ 1.1	16.6 $\pm$ 1.6
	HLA-B13	60.4 $\pm$ 4.6	25.3 $\pm$ 2.3
	B38	41.1 $\pm$ 2.9	10.6 $\pm$ 0.6
	B44.2	54.4 $\pm$ 2.1	21.1 $\pm$ 1.2
	B49	41.1 $\pm$ 3.8	11.5 $\pm$ 1.2
	B51	41.7 $\pm$ 0.4	15.5 $\pm$ 1.2
III	untransfected CIR	26.1 $\pm$ 0.3	23.4 $\pm$ 0.5
	HLA-Bw58	39.6 $\pm$ 4.7	8.1 $\pm$ 2.1
	HLA-B7.1	20.2 $\pm$ 0.8	5.7 $\pm$ 1.2
	H-2D <sup>p</sup>	60.2 $\pm$ 9.7	11.7 $\pm$ 2.8
	H-2K <sup>b</sup>	86.7 $\pm$ 6.0	8.8 $\pm$ 5.9
	untransfected CIR	17.9 $\pm$ 2.2	14.1 $\pm$ 1.5

All transfectants were assayed as described in Fig. 1 legend. Results of three experiments are shown. The binding of class I transfected and untransfected CIR cells to CD8 expressing (CD8<sup>+</sup>) and non-expressing (CD8<sup>-</sup>) CHO cells is shown. Only HLA-Aw68.1 and HLA-Aw68.2 reproducibly showed no specific binding to CD8. The significance of variation in the positive binding of the other class I molecules is not known; it may result from differences in the properties of the individual transfectants and/or the affinity of the class I–CD8 interaction.

molecules, H-2K<sup>b</sup> and H-2D<sup>p</sup> (Table 1). These results indicate conservation of the CD8 binding site in class I molecules of humans and mice. It was therefore surprising to find that two human class I molecules; HLA-Aw68.1 and -Aw68.2, showed no specific interaction with CD8 in the cell–cell binding assay (Table 1). This effect cannot be attributed to differing levels of HLA-A expression by the various transfectants (Fig. 1) and most probably results from differences in their primary structures. Fortunately, HLA-Aw68.1 and -Aw68.2 are closely related in amino-acid sequence to molecules such as HLA-A2.1 and HLA-Aw69 which do bind CD8. For example, HLA-Aw69 and HLA-Aw68.1 differ at only six positions in the  $\alpha_2$  domain and one position (245) in the  $\alpha_3$  domain<sup>4</sup>. We therefore reasoned that one or more of these substitutions is responsible for the difference in CD8 binding. Furthermore, a comparison of over 50 HLA-A,B,C sequences identified 245 as the only position at which there is a substitution that is specific to HLA-Aw68.1 and -Aw68.2 (ref. 4 and unpublished observations). In these two molecules, Val occurs at position 245 whereas other HLA-A,B,C molecules have Ala. These considerations suggested the involvement of residue 245 and the  $\alpha_3$  domain in the CD8 binding site.

To test this hypothesis we made a pair of mutants: the Ala 245 of HLA-A2.1 was converted to Val, giving the A2.1m245 mutant; and the Val 245 of HLA-Aw68.1 was converted to Ala, giving the Aw68.1m245 mutant. Analysis of transfectants expressing these mutant genes showed that the CD8 binding phenotype correlated with the residue at position 245 (Fig. 1). Thus mutant A2.1m245 showed no binding to CD8 whereas mutant Aw68.1m245 bound CD8 equivalent to wild-type HLA-A2.1. The specificity of this binding was also identical to that obtained with wild-type HLA-A2.1, in that it was dependent on CD8 and could be inhibited by monoclonal antibodies against CD8 and class I HLA molecules. Results of similar experiments with various other mutants argue against the involvement of the  $\alpha_1$  and  $\alpha_2$  domains in binding CD8. In these mutants the six

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TABLE 2 CD8 binding to mutants of HLA-Aw69 and Aw68.1

HLA molecule	95	97	107	114	116	156	245	CD8 binding
Aw69	V	R	W	H	Y	L	A	+
69m95, 97, 245	I	M	W	H	Y	L	V	—
69m107, 245	V	R	G	H	Y	L	V	—
68.1m95, 97	V	R	G	R	D	W	V	—
68.1m107	I	M	W	R	D	W	V	—
Aw68.1	I	M	G	R	D	W	V	—

HLA-Aw69 and Aw68.1 differ only at the seven positions shown. Amino-acid substitutions in the HLA mutants are given by the standard single-letter code. Assays were performed as described in Fig. 1 legend.

differences (positions 95, 97, 107, 114, 116, 156) between HLA-Aw69, which binds CD8, and HLA-Aw68.1, which does not, were assessed. Conversion of various combinations of these substitutions from one sequence to the other had no effect on the CD8 binding phenotype, which in every case correlated with residue 245 (Table 2).

Previous speculation has suggested an Arg-Phe-Asp-Ser (RFDS) sequence, shared by many class I and class II MHC sequences and related to the fibronectin cell binding tetrapeptide Arg-Gly-Asp-Ser (RGDS), as a possible site for CD8 or CD4 binding<sup>5</sup>. Furthermore, Auffray *et al.* have shown that RFDS-containing peptides derived from class II sequences inhibit T-cell responses<sup>6</sup>. Conversely, the RFDS sequence of HLA-A2.1 (residues 35–38 of the  $\alpha_1$  domain) is, from the crystallographic structure, inaccessible and therefore an unlikely site for CD8 binding<sup>7,8</sup>. This view is supported by our finding that a mutant of HLA-A2.1 in which Arg 35 is substituted by Val shows no loss of CD8 binding activity (data not shown).

The question arises as to whether the differences in the cell-binding assay have functional consequences for cytotoxic T cells. To address this, we compared recognition of position 245 mutants by both alloreactive and antigen-specific, MHC-

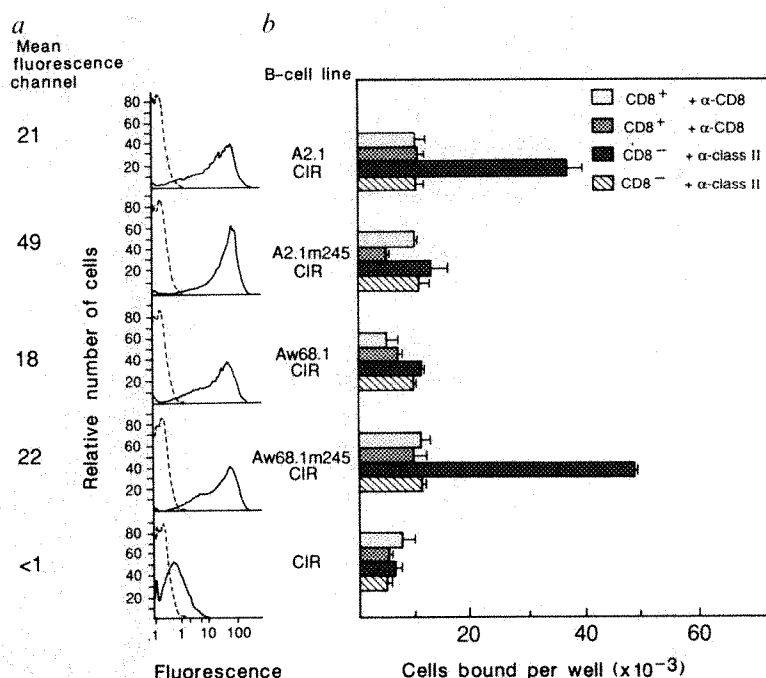
restricted CTL. The alloreactive CTL show specificity for HLA-A2.1 and effectively lyse the HLA-A2.1 transfectant. This lysis, however, is reduced by replacement of Ala 245 with Val (Fig. 2). By contrast, these CTL show a poor lysis of the HLA-Aw68.1 transfectant, which is considerably improved by replacement of Val 245 with Ala. Similar effects were observed with HLA-A2.1-restricted CTL lines specific for peptide 56–68 of the influenza matrix protein. Cytolysis was significantly reduced when Ala 245 of the HLA-A2.1 restriction molecule was replaced by Val (Fig. 2). Changes in CTL function can thus be correlated with changes observed in the cell-cell binding assay: greater lysis is observed when the target molecule binds more strongly to CD8.

Both alloreactive and antigen-specific CTL used in these experiments were inhibited by anti-CD8 monoclonal antibodies. Surprisingly, inhibition was not dependent upon whether the class I target could bind CD8 in the cell-cell binding assay. Thus recognition of HLA-Aw68.1 or HLA-A2.1m245 was inhibited by anti-CD8. In representative experiments, cytolysis by alloreactive CTL of HLA-Aw68.1 and HLA-A2.1 was, respectively, reduced from 29 to 8% and from 56 to 39% by anti-CD8 antibody 51.1. Lysis of peptide-sensitized A2.1m245 transfectants by influenza-specific CTL was reduced from 37 to 16% by anti-CD8, whereas no effect was seen on lysis of HLA-A2.1 transfectants. These observations might reflect a residual affinity of these molecules for CD8 that is not detected in the cell binding assay. An alternative is suggested by other studies in which the inhibition of CTL by anti-CD8 antibodies, in a manner that is unrelated to binding of class I molecules, is interpreted as evidence for a regulatory role for CD8 in T-cell activation<sup>9,10</sup>.

It is clear that substitution at position 245 alters the interaction of class I HLA molecules with CD8. This could result from a direct involvement of this residue in the interaction or by an indirect conformational effect on a distant site. The crystallographic structures of HLA-A2.1 and -Aw68.1 at high resolution have been determined and they are extremely similar (T. Garrett, M. Saper, P. Bjorkman and D. Wiley, unpublished observations). Thus, it is probable that the distinct functional properties

FIG. 1 Substitution at position 245 determines natural polymorphisms in CD8 binding. *a*, Expression of HLA-A2.1, A2.1m245, Aw68.1m245 and Aw68.1 molecules by transfectants of the CIR B-cell line which expresses no endogenous HLA-A,B molecules<sup>18</sup>. Indirect binding of the HLA-A2,A28-specific monoclonal antibody CR11-351 (ref. 19) (solid lines) was compared to an anti-actin control<sup>20</sup> (dotted lines) by flow cytometry. *b*, Binding of class I transfectants to CD8 expressing and non-expressing CHO cells in the presence of either anti-CD8 or anti-HLA-DR. Binding to CHO.1 and CHO.4 cells is labelled CD8<sup>+</sup> and CD8<sup>−</sup>, respectively. In each group of four results the combination of cells and antibodies is, going from top to bottom: CD8<sup>+</sup> CHO cells with anti-CD8 ( $\alpha$ -CD8); CD8<sup>−</sup> CHO cells with anti-CD8; CD8<sup>+</sup> CHO cells with anti-HLA-DR ( $\alpha$ -class II); CD8<sup>−</sup> CHO cells with anti-HLA-DR. The standard errors are indicated by the 'error' bars. The HLA-A2,A28-specific monoclonal antibody CR11-351 also inhibited cell-cell binding when preincubated with the CIR transfectants (data not shown). A second independently derived set of transfectants had identical CD8 binding characteristics (data not shown).

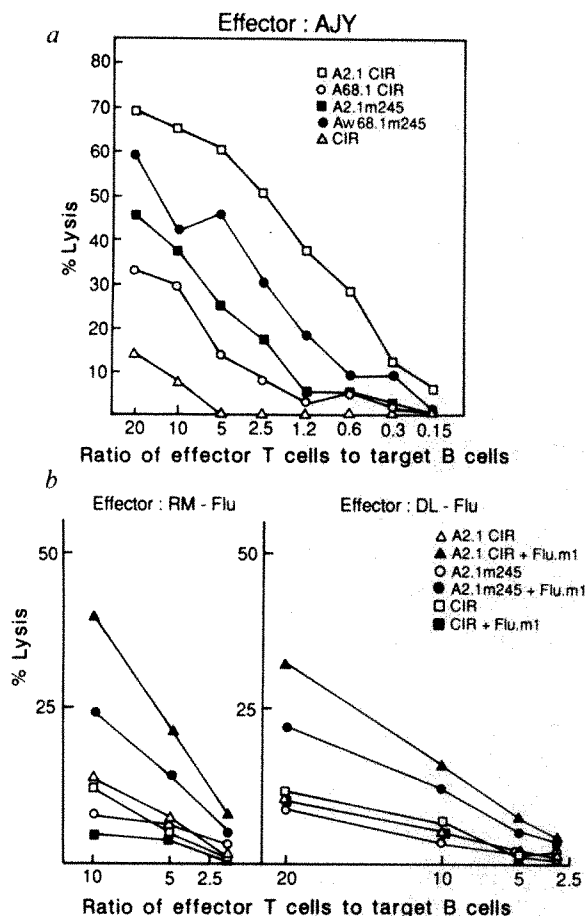
**METHODS.** Mutagenesis and transfection of class I genes were as previously described<sup>18</sup>. Flow-cytometric analysis of cells incubated first with monoclonal antibody and then with fluoresceinated goat anti-mouse antibody used a Becton-Dickinson FACS with a 10 $\times$  neutral density filter. In CD8 binding experiments, CIR transfectants with class I genes were radio-labelled with <sup>35</sup>S methionine, washed twice, resuspended in PBS-containing, 10% fetal calf serum, and incubated in 96-well plates with confluent monolayers of transfected CHO cells. The CHO cells were previously incubated (30 min, 20  $\mu$ g ml<sup>−1</sup>, 37 $^{\circ}$ ) either with monoclonal antibody 51.1 (ref. 21), which is specific for CD8, or CA206, which is specific for HLA-DR (ref. 22), and was used as an irrelevant antibody control. CHO.1



cells express no CD8 whereas CHO.4 cells express ~160-fold more CD8 than peripheral T cells<sup>3</sup>. After incubation at 37 $^{\circ}$  for 1 hour, wells were washed extensively, and bound radioactivity measured.



of these two molecules arise from the nature and disposition of the side-chains at the positions of amino-acid substitution and not through gross conformational differences. These observations combined with the complete change in CD8 binding phenotype caused by single substitutions at position 245 in HLA-A2.1 and HLA-Aw68.1 strongly favour the direct involvement of this residue and the  $\alpha_3$  domain in the CD8 binding site.



**FIG. 2** Cytolysis of class I HLA-expressing CIR transfectants by alloreactive and antigen-specific T cells. **a**, Lysis of CIR transfectants expressing HLA-A2.1, A2.1m245, Aw-68.1m245 and Aw68.1 by an HLA-A2 alloreactive CTL culture AJY. In some experiments, the A2.1m245 transfectants were lysed better than Aw68.1m245-bearing cells. In all experiments, however, the AJY culture recognized HLA-A2.1 more effectively than A2.1m245, and Aw68.1m245 more effectively than HLA-Aw68.1. **b**, Lysis by influenza-specific CTL of HLA-A2.1- and A2.1m245-expressing transfectants after incubation with peptide corresponding to residues 56–68 of the influenza matrix protein. Donor RM (HLA-A1,A2; Bw55, B44), left panel; donor DL (HLA-A2, A11; B27, B44), right panel. All but one of the responders lysed HLA-Aw68.1- or Aw68.1m245-bearing transfectants.  $\Delta$ ,  $\triangle$ : lysis of A2.1 transfectant with and without the Flu.m1 peptide, respectively.  $\bullet$ ,  $\circ$ : lysis of A2.1m245 transfectant with and without the Flu.m1 peptide, respectively.  $\blacksquare$ ,  $\square$ : lysis of untransfected CIR cells with and without the Flu.m1 peptide, respectively. **METHODS.**  $^{51}\text{Cr}$ -release assays were performed as previously described<sup>23</sup>. The AJY culture was generated by *in vitro* stimulation of peripheral blood lymphocytes (PBL) from a single donor AK (HLA-A3; B7, B38) with the HLA-A2.1-expressing cell line JY (ref. 23), and it lyses HLA-A2.1-, Aw69-, and Aw68-bearing targets with varying efficiency. Influenza-specific cultures were generated essentially as previously described<sup>24</sup>. PBL from six HLA-A2 positive donors were stimulated with  $10 \mu\text{g ml}^{-1}$  of a peptide (Flu.m1) derived from residues 56–68 of the influenza matrix protein shown previously to generate HLA-A2-restricted CTL (ref. 25). Three days after the initial stimulation, supernatant containing growth factors from IL-2-stimulated PBL was added. Cultures were restimulated after 7 days to generate secondary cultures. For cytolysis assays, target cells were preincubated overnight with  $10 \mu\text{g ml}^{-1}$  of the Flu.m1 peptide, then used in  $^{51}\text{Cr}$ -release experiments.

Further support for this interpretation comes from experiments showing that a mutant of H-2D<sup>d</sup> derived *in vitro* with a single substitution at position 227 shows specific loss of interaction with cytotoxic T cells that are dependent on Lyt-2, the murine equivalent of CD8<sup>11,12</sup>. Positions 227 and 245 of the  $\alpha_3$  domain are located proximal to the membrane attachment site in a solvent accessible surface region and are separated by only 10.5 Å (refs 7, 8). Both could therefore contribute to the CD8 binding site.

Davis and Bjorkman have raised the possibility that CD8 interacts with the  $\alpha_1$  and  $\alpha_2$  domains of class I MHC molecules, in a manner analogous to the T-cell receptor<sup>13</sup>. A consequence of this model is that simultaneous interaction of CD8 and the T-cell receptor with the same class I MHC molecule cannot occur. Our results and those of Potter *et al.*<sup>11,12</sup>, suggesting interaction of CD8 with the  $\alpha_3$  domain, do not support this view and leave open the possibility that a complex of T-cell receptor and CD8, binding to the same class I MHC molecule (plus its antigenic peptide), is crucial to T-cell activation.

Comparison of the amino-acid sequences of antigen-presenting class I MHC molecules from seven mammalian species (summarized in ref. 14) shows that HLA-Aw68.1 and -Aw68.2 are unique in not having Ala at position 245. This combined with the observation that all 15 molecules with Ala at this position bound CD8 in the cell-cell binding assay suggest that the non-binding CD8 phenotype will be rare among class I MHC products. In most human populations, however, the frequency of HLA-A28, of which HLA-Aw68.1 and -Aw68.2 are subtypes, is 8–9% (ref. 15). The intriguing question is whether reduced CD8 binding confers any selective advantage or disadvantage to individuals expressing these molecules. For example, does it affect the capacity of HLA-Aw68.1 and -Aw68.2 to present antigens, or the repertoire of HLA-Aw68.1- and HLA-Aw68.2-restricted T-cell receptors selected in the thymus? In studies by Rickinson *et al.* CTL specific for Epstein-Barr virus (EBV), generated by *in vitro* priming and restricted to HLA-Aw68, were never obtained, although HLA-Aw69-restricted CTL lysed infected HLA-Aw68 targets<sup>16,17</sup>. This suggests that although HLA-Aw68 can present EBV peptides to specific CTL, it may be deficient in generating such a response. The number of epitopes and restriction patterns described for human class I MHC molecules, however, is still too small to assess the relative use of HLA-Aw68.1 and -Aw68.2.  $\square$

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# Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification

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THE molecular analysis of many genetic diseases requires the isolation of probes for defined human chromosome regions. Existing techniques such as the screening of chromosome-specific libraries<sup>1</sup>, subtractive DNA cloning<sup>2</sup> and chromosome jumping<sup>3</sup> are either tedious or not generally applicable. Microdissection and microcloning has successfully been applied to various chromosome regions in *Drosophila* and mouse<sup>4-9</sup>, but conventional microtechniques are too coarse and inefficient for analysis of the human genome<sup>10-12</sup>. Because microdissection has previously been used on unbanded chromosomes only, cell lines in which the chromosome of interest could be identified without banding had to be used. At least one hundred chromosomes were needed for dissection and  $\lambda$  vectors used to achieve maximum cloning efficiency. Recombinant phage clones are, however, more difficult to characterize than plasmid clones. Here we describe the dissection of the Langer-Giedion syndrome region on chromosome 8 from GTG-banded metaphase chromosomes (G-banding with trypsin-Giemsa) and the universal enzymatic amplification of the dissected DNA. Eighty per cent of clones from this library (total yield 20,000) identify single-copy DNA sequences. Fifty per cent of clones detect deletions in two patients with Langer-Giedion syndrome. Although the other clones have not yet been mapped, this result demonstrates that thousands of region-specific probes can be isolated within ten days.

We have isolated probes for the Langer-Giedion syndrome chromosome region (8q24.1) (refs 13, 14). Deletion of a set of unknown genes within this region leads to sparse hair, lax skin, bulbous nose, cone-shaped epiphyses, multiple cartilaginous exostoses and mental retardation<sup>15</sup>. Using an electronically controlled micromanipulator, we dissected the distal third of band 8q23 and the proximal two-thirds of sub-band 8q24.1 from normal GTG-banded chromosomes (Fig. 1). The dissected

region is estimated to comprise approximately 10,000 kilobases (kb). As the distal extent of the Langer-Giedion syndrome chromosome region is uncertain, we included chromosome material distal to the deletions in our two patients (see below and Fig. 3). To facilitate the identification and dissection of the chromosomes, the dissection was not performed in an oil chamber, and we used straight needles and pipettes, rather than microforged ones.

The dissected chromosome fragments were transferred to a collection drop kept in a moist chamber. After the dissection of 37 chromosomes, the microdrop was overlaid with oil. Chromosomal DNA (estimated yield, 0.5 pg) was digested with the restriction enzyme *RsaI* and ligated to a *SmaI*-cut pUC plasmid (Fig. 2). The inserts were then amplified by the polymerase chain reaction using the universal M13/pUC sequencing and reverse sequencing primers. In contrast to the amplification of specific DNA sequences in genomic DNA<sup>16</sup>, the universally primed polymerase chain reaction allows the simultaneous amplification of many different unknown DNA sequences. After 26 cycles of denaturation, annealing and DNA synthesis, the amplified inserts were released by *EcoRI* digestion and cloned into pUC13. We obtained 20,000 clones, of which we characterized 50 in detail.

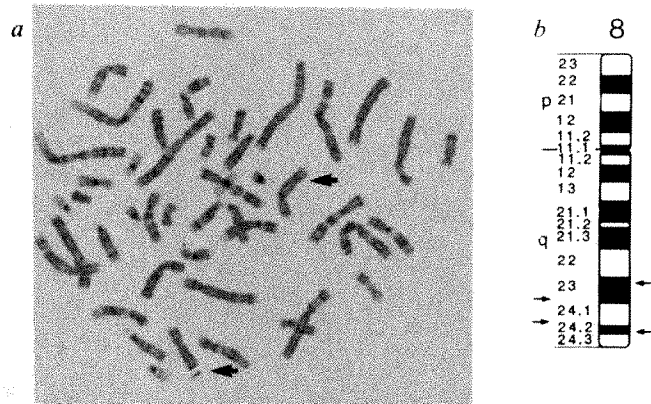
The insert size ranged from 52 base pairs (bp) to 350 bp (mean, 150 bp). This is smaller than the mean size of *RsaI* restriction fragments (495 bp)<sup>17</sup>, probably because of the preferential amplification of small inserts. The cloning of small fragments has the advantage of yielding a high percentage of repeat-free clones: 10 inserts (20%) contained repetitive DNA and 40 inserts (80%) identified single-copy fragments. All but two of the single-copy clones were different. These clones were used to probe Southern blots containing DNA from two Langer-Giedion patients with a visible chromosome deletion.

Patient S.H. (ref. 18) has a deletion of band q23 and sub-band q24.11 (ref. 13); patient T.T. (ref. 19) has a deletion of the distal half of band q23 and the proximal half of sub-band q24.1 (ref. 13). Twenty clones (50%) map outside the deletions in S.H. and T.T. These clones are probably derived from the dissected material distal to the deletion in T.T., but cannot be assigned at present. Eleven clones are deleted in both patients, and nine clones are deleted in patient T.T. only (Fig. 3). These clones are currently being extended by chromosome walking and physically linked by pulsed-field gel electrophoresis. None of the microclones maps to the region deleted in S.H. only, which is proximal to the dissected region. This demonstrates the precision of our technique.

In addition to the region on chromosome 8, we have successfully dissected and cloned DNA from 11p13 (Wilms tumour-aniridia) and 15q11-12 (Prader-Willi syndrome). In these experiments, only 20 chromosomes were dissected and all of the clones tested so far map to the dissected bands. If desired,

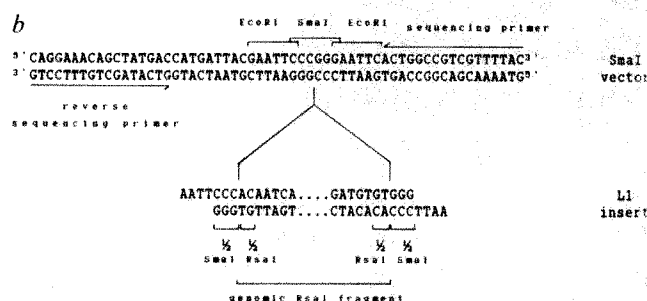
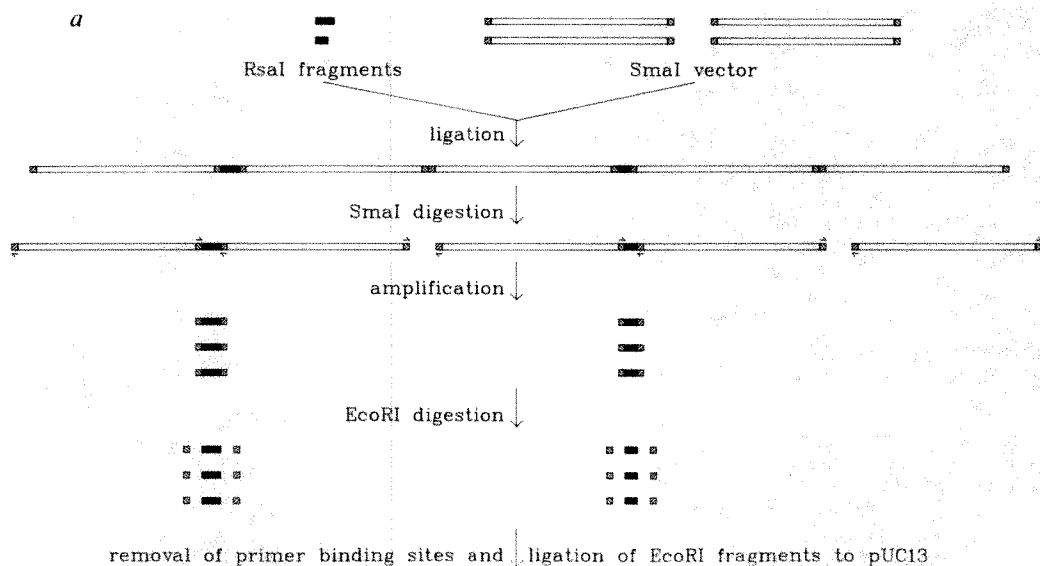
FIG. 1 Microdissection of chromosome 8. a, Human GTG-banded metaphase with two chromosomes 8, one dissected (8q23.3→8q24.21) and one not, for comparison. b, Diagram showing a normal G-banding pattern of a human chromosome 8 (ref. 20). The arrows on the left indicate the region dissected in all chromosomes 8. The arrows on the right mark the most proximal and most distal limits of the various cuts.

METHODS. Cells from normal amniotic fluid cell cultures were collected using the pipette method (time of fixation was 10–20 s)<sup>21</sup>. The chromosomes were spread onto clean coverslips, washed in 70% ethanol, air dried and GTG-banded. Microdissection was performed on an inverted microscope (IM Zeiss; magnification, 1,200 $\times$ ) with the aid of a micromanipulator (MR MOT; Zeiss) and extended siliconized glass needles. The dissected pieces ( $n=37$ ) were pooled into a 1-ml collection drop (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 0.1% SDS, 1% glycerol, 500  $\mu\text{g ml}^{-1}$  proteinase K) which was situated in a small moist chamber on a separate siliconized coverslip. Proteinase K digestion and phenol extractions were performed under oil essentially as described<sup>9</sup>. The chromosomal DNA was digested with *RsaI* (Boehringer) at 37 °C for 2.5 h (final concentration 6 units  $\mu\text{l}^{-1}$ ). Another aliquot of *RsaI* was added, and the mixture incubated for another 2.5 h. The enzyme was inactivated by three phenol extractions.

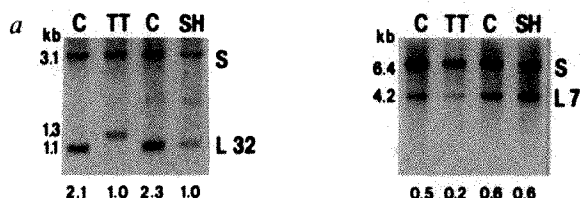


**FIG. 2 Universal DNA amplification procedure.** *a*, *Rsa*I fragments (filled bars) are ligated to a fivefold molar excess of a *Sma*I-cut pUC vector (open bars) that contains a single *Sma*I site flanked by two *Eco*RI sites (see polylinker sequence in *b*). Polyethylene glycol is included in the ligation mixture to obtain long concatemers<sup>22</sup>. Nonrecombinant polylinker sequences are recut with *Sma*I, and inserts are amplified with the help of DNA polymerase and the universal M13/pUC sequencing (←) and reverse sequencing primer (→). After removal of the primer binding sites (hatched bars) by *Eco*RI digestion and gel filtration, the amplified fragments are cloned into pUC13. *b*, Polylinker sequence of the *Sma*I vector and insert sequence of clone L1. The *Sma*I vector is a pUC derivative with a single *Sma*I site flanked by *Eco*RI sites. It allows the ligation of *Rsa*I fragments into the *Sma*I site and the release of the amplified inserts by *Eco*RI digestion. The released fragments are cloned into the *Eco*RI site of pUC13 (Pharmacia). As shown for one example (clone L1), the clone inserts contain half a *Sma*I and half a *Rsa*I site at both ends.

**METHODS.** A synthetic *Eco*RI-*Sma*I adaptor (Pharmacia) was inserted between the two *Eco*RI sites of M13mp7 (BRL) to yield the symmetric polylinker sequence *Eco*RI-*Sma*I-*Eco*RI. The 320-bp *Pvu*II fragment spanning the polylinker sequence of this M13 derivative was then used to replace the respective *Pvu*II fragment of pUC19 to yield the *Sma*I vector. Chromosomal *Rsa*I fragments (estimated yield 0.5 pg) were ligated to 13.5 pg *Sma*I vector in the presence of 15% polyethylene glycol (relative molecular mass 8,000). T4 DNA ligase (New England Biolabs; 0.08 units) was added, and the ligation was carried out at 15 °C overnight. The reaction mixture (128 nl) was then taken up into 2 µl of water and transferred to a microfuge tube. After heat inactivation of the ligase, non-recombinant polylinker sequences were recut with 2 units of *Sma*I (Boehringer) at 25 °C for 1 hour (final volume 5 µl). The sample was adjusted to 50 µl of 20 mM Tris-HCl pH 7.8, 5 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 2.5 mM of each of the four deoxynucleoside triphosphates and 1 µM each of the M13/pUC sequencing and reverse sequencing primers. The DNA was denatured by incubation at 95 °C for 5 min, and the primers were allowed to anneal at 33 °C for 2 min. After the addition of 1 unit of Klenow DNA polymerase (MEDAC, Hamburg) in 1 µl amplification

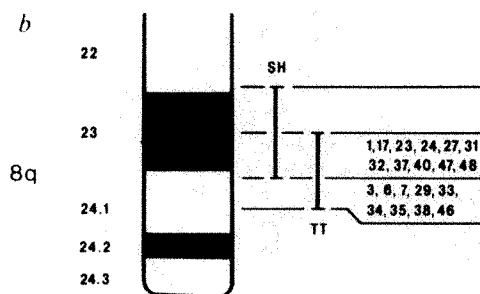


buffer, DNA synthesis was carried out at 37 °C for 10 min. This cycle was repeated 25 times, except that subsequent denaturations were performed at 92 °C for 2 min, and the time for DNA synthesis was reduced to 5 min after the fifth cycle. After the last primer extension step, Klenow DNA polymerase was heat-inactivated and the amplification products were digested with *Eco*RI. The amplified fragments were purified by gel filtration through two successive Sephadryl S-200 spun columns (Pharmacia) and ligated to dephosphorylated *Eco*RI-cut pUC13 (Pharmacia). The ligation products were used to transform competent DH5α cells (BRL). Recombinant plasmids were purified on Qiagen-20 tips. DNA sequences were determined by the dideoxy chain-termination method<sup>23</sup>.



**FIG. 3 Chromosomal localization of the microclones.** *a*, Quantitative Southern blot analysis of Langer-Giedion syndrome patients. Genomic DNA from a normal control (C) and from the patients S.H. and T.T. was digested with *Hind*III and probed with the microclones indicated beside the bands. Hybridizations were performed essentially as described previously<sup>24</sup>. Various probes that map outside the deletions served as an internal hybridization standard (S). Each lane contains approximately the same amount of DNA. The relative intensity of the autoradiographic signals L32:S and L7:S was determined using a Shimadzu CS9000 densitometer and is given beneath each lane. Clone L32 is deleted in S.H. and T.T., and clone L7 is deleted in T.T. only. Similar results were obtained in two other independent experiments. The

the number and the size of the dissected fragments could be reduced much further, but the number of amplification cycles would have to be increased. The universal DNA amplification system also allows the cloning of minute amounts of DNA from ancient tissue samples and the construction of random primed cDNA libraries from small cell numbers.



larger L32 band in T.T. represents a rare allele of a *Hind*III restriction fragment length polymorphism<sup>25</sup>. *b*, All microclones were mapped by quantitative Southern blot hybridizations as shown above. In each case, three independent experiments were performed. The distal part of 8q and the deleted chromosome segments in S.H. and T.T. are shown. The numbers on the right refer to the clones found to be deleted in both patients and in T.T. only.

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## Isotype exclusion and transgene down-regulation in immunoglobulin- $\lambda$ transgenic mice

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A GIVEN B lymphocyte makes an antibody containing either  $\kappa$ - or  $\lambda$ -light chains, but not both<sup>1,2</sup>. This isotype exclusion is effected at the level of the rearrangement of the immunoglobulin gene segments<sup>3–5</sup>, although by an unknown mechanism. An attractive possibility is that, following productive rearrangement of one of the light-chain loci, the newly synthesized light-chain polypeptide inhibits DNA rearrangement for the other isotype. To test such feedback regulation, we have created transgenic mice carrying a rearranged  $\lambda_1$ -gene. By contrast with the B cells in normal newborn mice which are mainly  $\kappa^+ \lambda^-$ , the B cells in the newborn transgenic mice express  $\lambda$ - but not  $\kappa$ -chains. We propose that the synthesis of any light chain, be it  $\kappa$  or  $\lambda$ , that allows expression of IgM on the cell surface results in a cessation of all V–J joining. Interestingly, the limited light-chain repertoire of the transgenic mice does not persist and most adult B cells express endogenous  $\kappa$ -rearrangements and down-regulate the transgene.

Analysis of transgenic mice has provided strong evidence for a role for immunoglobulin polypeptides in mediating a feedback regulation of allelic exclusion<sup>6–10</sup>. Thus, a transgenic  $\kappa$ -gene inhibits rearrangement of the endogenous  $\kappa$ -loci<sup>7,8</sup>. It has not been established, however, whether the  $\kappa$ -transgene has a role in mediating isotype exclusion as, even in normal mice, rearrangement of the  $\lambda$ -loci is a rare event. We have therefore approached this problem by creating  $\lambda$ -transgenic mice to see whether the  $\lambda$ -transgene exerts an effect on rearrangement of the endogenous  $\kappa$ -loci. A plasmid was assembled that contains a rearranged mouse  $\lambda_1$ -gene linked to a chimaeric  $\alpha 2$ -heavy-chain gene (Fig. 1a). This  $\alpha 2$  gene lacks the exons encoding the membrane-anchoring sequences and the presence of this human  $\alpha 2$ -gene allowed unambiguous identification of mouse cells expressing the transgene. Transcription of both the heavy- and light-chain genes is potentiated by the mouse IgH enhancer.

Linearized plasmid DNA was injected into mouse eggs. Of 42 mice born, 12 carried integrations of the injected DNA. Nine

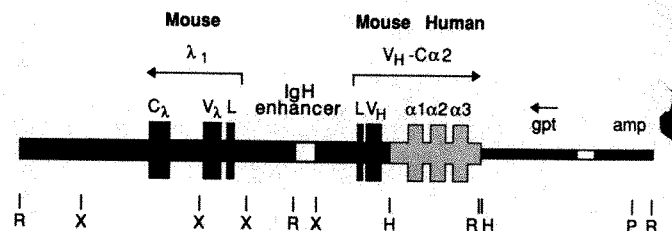


FIG. 1 Structure of plasmid DNA: thick filled lines; mouse immunoglobulin DNA; hatched lines, human immunoglobulin DNA; open boxes, IgH and SV40 enhancers; thin filled lines, pSV2gpt vector. Restriction sites are abbreviated: R, EcoRI; X, XbaI; H, HindIII; P, PvuII. The plasmid is a derivative of pSV-V<sub>H</sub>- $\alpha 2$  described previously<sup>21</sup> and contains a 7.4-kb EcoRI fragment including the rearranged  $\lambda_1$  gene of the mouse HOPC2020 plasmacytoma<sup>22</sup>. Plasmid DNA was linearized at the PvuII site in the vector and transgenic mice were derived as previously described<sup>23</sup>.

of these transgenic founder mice expressed the transgene as judged by enzyme-linked immunosorbent assays (ELISA) which detected the presence of IgA2, $\lambda_1$  anti-NP antibody in serum. Northern blot analysis (data not shown) revealed that expression of the transgene was lymphoid-specific. Most analyses were performed on descendants of founders 8,023 (which gave serum titres of  $\sim 5 \mu\text{g ml}^{-1}$  at 4 weeks of age) or 8,032 (serum titres of  $100\text{--}200 \mu\text{g ml}^{-1}$ ).

Analysis of cells from the offspring of founder 8,032 using the fluorescence-activated cell sorter (FACS) showed that the transgenic mice and their non-transgenic siblings contained similar proportions of IgM<sup>+</sup> B cells in their spleens (Fig. 2a). The newborn transgenic mice, however, differ radically from their littermates with respect to immunoglobulin light-chain expression. The transgenics have few  $\kappa$ -bearing B cells, most carrying  $\lambda$ -chains on the surface (Fig. 2a). Cytoplasmic immunofluorescence analysis gave similar results (Table 1) and revealed that most of the  $\lambda^+$ -cells stained for human  $\alpha 2$ , indicating that they were likely to be expressing the transgene rather than an endogenous  $\lambda$ -gene. By contrast with the non-transgenic siblings, however, the isotype exclusion in the transgenic mice, although impressive, is far from absolute, with  $\sim 10\%$  of the B cells making both  $\kappa$ - and  $\lambda$ -chains (Table 1). The  $\alpha 2$ -heavy-chain itself was detected only intracytoplasmically.

Transgene expression among splenic B cells of adult transgenic mice contrasts strikingly with that in the neonates. The proportion of  $\lambda$ -expressing cells decreases considerably with age and there is a corresponding increase in the number of B cells that express  $\kappa$ -light chains (Fig. 2a and Table 1). This fall with age in the proportion of transgene-expressing cells is observed in F<sub>1</sub> and subsequent generation offspring of both the 8,023 and 8,032 founders. We believe that a small proportion

TABLE 1 Cytoplasmic immunofluorescence analysis of immunoglobulin light-chain expression

	Transgenic		Control	
	Neonates	Adults	Neonates	Adults
$\lambda$ -only	73	25	17	3
$\kappa$ , $\lambda$ -doubles	10	9	0	0
$\kappa$ -only	17	66	83	97

Results in each column show the percentage of total light-chain-positive spleen cells from pools of 3 mice of 8,032 offspring which type as  $\kappa$ -only,  $\lambda$ -only or  $\kappa$ ,  $\lambda$ -doubles. Neonatal mice were 4 days old and adults 9 weeks old. Immunofluorescence on methanol-permeabilised cells was carried out using a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse  $\lambda$ -antisera (Southern Biotechnology) and biotinylated goat anti-mouse  $\kappa$ -antisera (Amersham) with phycoerythrin-conjugated streptavidin. It is notable that in the strain of mice used here (C57BL/6  $\times$  CBA F<sub>1</sub>), the proportion of  $\lambda^+$  cells in non-transgenic neonates is somewhat higher than that reported previously<sup>25</sup> for BALB/c and C57BL/6.

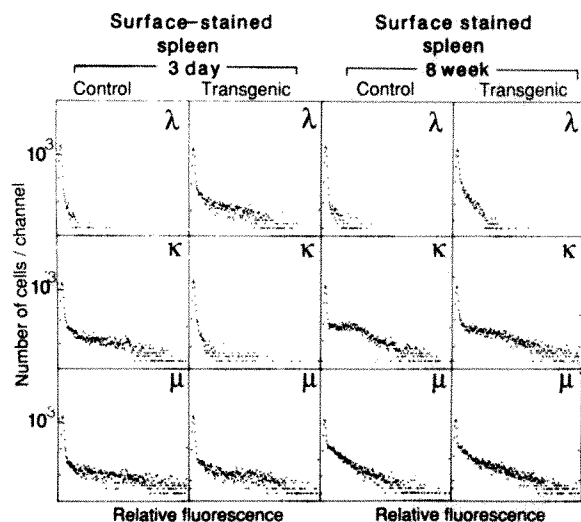


FIG. 2 Fluorescence analysis of transgene expression. FACS profiles of spleen cells surface-labelled with biotinylated sheep anti-mouse  $\kappa$ ,  $\lambda$  or  $\mu$  antibodies and fluorescein-conjugated streptavidin; dead cells were gated out by scatter gating. Samples were obtained from 3 day or 8-week-old offspring of 8.032. The results shown here are from four individual mice although similar profiles were obtained from other siblings using both neonatal and adult  $F_2$  offspring of both 8.023 and 8.032.

of B cells either fails to activate or, alternatively, down-regulates expression of the transgene; these cells then undergo rearrangement of the endogenous light-chain loci, and selection at the cellular level results in the temporal change in antibody expression<sup>11</sup>.

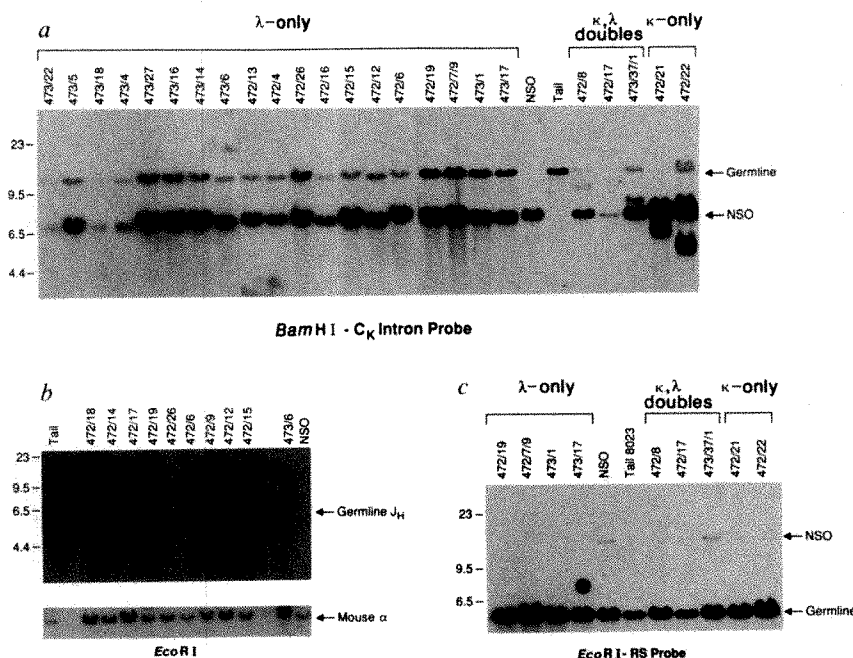
Hybridomas were established to ascertain whether the inhibition of  $\kappa$ -light-chain expression in the transgenic mice was due to an inhibition of  $\kappa$ -gene rearrangement. Spleen cells from 3.5- or 8-week-old transgenic mice were fused with the NS0 plasmacytoma. DNA was prepared from 22 randomly chosen cloned hybridomas that secreted  $\lambda$ -light chains. Of these 22 clones, three turned out to be  $\kappa$ , $\lambda$ -double producers with the other 19 making  $\lambda$  as their sole light chain. Southern blot analysis (Fig. 3a) revealed that the  $\kappa$ -loci of spleen cell origin in the  $\lambda$ -only hybrids were always in the germline configuration, in stark contrast to those from  $\lambda$ -expressing B cells of non-transgenic

mice which normally bear aberrant  $\kappa$ -rearrangements on one or both alleles<sup>3-5,12</sup>. Furthermore, the state of the  $\kappa$ -loci in the  $\lambda$ -hybridomas from the transgenic mice also contrasts with the  $\kappa$ -loci in random hybridomas made by fusing spleen cells from normal (CBA  $\times$  C57BL/6) $F_1$  mice with NS0. In this case, DNA was analysed from randomly chosen, cloned hybridomas whether or not they produced immunoglobulin. Consistent with previous observations on primary cells<sup>13</sup>, we found that the incoming  $\kappa$ -gene was rearranged in 9 out of 20 of these hybrids (data not shown). These results show therefore that the presence of the IgA2, $\lambda_1$  transgene leads to an inhibition of  $\kappa$ -gene rearrangement.

The mechanism regulating the hierarchy of rearrangements at the light-chain loci remains a mystery. Apart from aberrant  $V_{\kappa}$ - $J_{\kappa}$  joining, most  $\lambda$ -expressing hybridomas contain rearrangements involving the rearranging sequence element (RS)<sup>14,15</sup>. This element, which in man is termed  $\kappa$ -deleting element (kde)<sup>16,17</sup>, is located ~24 kilobase (kb) downstream of the  $C_{\kappa}$  exon and recombines with the joining signals belonging to a  $V_{\kappa}$  segment or in the vicinity of the  $J_{\kappa}$  elements with resultant destruction of the  $\kappa$ -locus. The significance of this RS recombination is not known. It has been proposed that it acts as a signal to initiate rearrangement at the  $\lambda$ -loci<sup>14</sup>. It is also possible, however, to envisage models in which productive  $\lambda$ -rearrangement triggers RS recombination and thereby ensures isotype exclusion. To test this second model, we looked at the status of the RS element in the  $\lambda$ -expressing hybridomas from the transgenic mice. Two main bands hybridizing with the RS probe are clearly visible (Fig. 3c); the strong 6-kb *Eco*RI fragment containing the germline RS element and a weak 13-kb band containing the rearranged RS of NS0. Several of the hybrids have lost the rearranged RS of the fusion partner, presumably through chromosome loss although we have no simple explanation for the fact that loss of the NS0, rearranged RS allele is particularly evident in the hybrids that express only  $\lambda$ -light chains. There is no evidence, however, of additional RS rearrangements in most of the hybridomas although one (NW473/22, a  $\lambda$ -only expresser, not shown) contains extra bands that hybridize weakly with the RS probe. On the basis of these results, it seems improbable that RS recombination is specifically activated by the presence of  $\lambda$ -light chains. Therefore, if RS recombination does indeed have a role in isotype exclusion it is more likely to lie in the activation of  $\lambda$ -rearrangement rather than in enabling  $\lambda$ -chains to switch off  $\kappa$ -rearrangement.

FIG. 3 Southern blot analysis of  $\kappa$ , IgH and RS rearrangements in splenic hybridomas. a,  $\kappa$ -rearrangements: DNA digested with *Bam*HI was probed with an *Xba*I-*Hind*III fragment from the mouse  $J_{\kappa}$ - $C_{\kappa}$  intron. The bands labelled NS0 are from the NS0 fusion partner and Germline indicates the band corresponding to the germline, unrearranged  $\kappa$  locus. b, IgH rearrangements: DNA was digested with *Eco*RI and probed with a 0.6-kb *Sac*I-*Bsu*36I fragment from between D-Q52 and  $J_{\mu}$ 1. The blot was then reprobed for the mouse immunoglobulin  $\alpha$ -heavy-chain gene so as to provide a control for DNA loading. c, RS rearrangements: DNA was digested with *Eco*RI and probed with an 0.8-kb *Sau*3A fragment (probe rs0.8 of ref. 15) for RS sequences. N indicates the rearranged RS element of the NS0 fusion partner and G indicates the germline-configuration RS band.

**METHODS.** The hybrids were derived by fusing spleen cells from an 8-week-old offspring of 8.023 (NW472 hybrids) or a 3.5-week-old offspring of 8.032 (NW473 hybrids) with NS0 plasmacytoma cells. After growth in selective medium, hybrids were cloned by limiting dilution.



Apart from the inhibition of  $\kappa$  rearrangement, the transgenic mice also exhibit a decreased amount of rearrangement at the IgH loci. Of a total of 25 random  $\lambda^+$ -hybrids examined from the transgenic mice, 13 (52%) contained a germline  $J_H$  allele as detected using a probe for the region located between D-Q52 and  $J_H1$  (one of the blots is shown in Fig. 3b). This is a considerably higher figure than would be expected from an analysis of IgH rearrangements both in primary B cells<sup>18</sup> as well as in random hybrids generated by fusing spleen cells from (C57BL/6  $\times$  SJL) mice with X63.Ag8.653 (ref. 10). We cannot formally exclude the possibility that this inhibition is due to the secreted form of the human  $\alpha 2$  heavy-chain polypeptide. A similar inhibition of IgH rearrangement, however, has recently been described in mice transgenic for a  $\kappa$ -light-chain gene where 22% of the hybrids were found to contain a germline  $J_H$  allele<sup>19</sup>. By analogy with the proposal of Manz *et al.*<sup>19</sup>, we suspect that the increased frequency of germline  $J_H$  loci in the hybridomas from the IgA2, $\lambda 1$  transgenic mice is probably caused by a combination of transgenic  $\lambda 1$  chain with the endogenous  $\mu_m$ -polypeptide leading to an early switch off of recombinase activity in pre-B-cells that have just rearranged their endogenous IgH locus. This could then lead to a diminished amount of D- $J_H$  rearrangement on the excluded IgH allele. The fact that we see a higher proportion of germline  $J_H$  alleles in the IgA2, $\lambda 1$  transgenic mice than has been described in the  $\kappa$ -transgenics could be due to that fact that expression of the  $\lambda 1$  transgene is potentiated by the IgH enhancer; it may therefore be switched on earlier than the  $\kappa$  transgene. Because of this inhibition of both heavy and light chain rearrangement, it is not surprising that the transgene causes an overall depression of B-cell development as the number of spleen cells in the transgenic mice was  $\sim 70\%$  that of their non-transgenic siblings.

The analysis of immunoglobulin light-chain gene expression in mouse plasmacytomas and in hybridomas from mice transgenic for a  $\kappa$ -light chain<sup>7,8,12</sup> supports a model in which  $V_\kappa$ - $J_\kappa$  joining is switched off following a signal delivered by a complete IgM, $\kappa$  antibody molecule. Recent data obtained using both transgenic mice and pre-B-cell lines were consistent with the membrane form of the IgM antibody mediating this feedback regulation<sup>9,20</sup>. From the work described here it is clear that a transgenic  $\lambda$ -light chain (presumably in association with the endogenous  $\mu_m$ -polypeptide) causes an inhibition of  $\kappa$ -locus rearrangement. It is probable that, following the production of intracellular  $\mu_m$  polypeptide chains, any light-chain rearrangement be it  $\lambda$  or  $\kappa$  that leads to IgM on the cell surface results in a cessation of all V-J joining. It is attractive to speculate that whereas the presence of  $\mu_m$ -polypeptide in the membrane of the endoplasmic reticulum signals a stop to heavy-chain rearrangement and a start to light-chain rearrangement, productive light-chain rearrangement causes the  $\mu_m$ -polypeptide to be translocated to the plasma membrane where it signals to stop all V-gene rearrangement. □

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## A dominant control region from the human $\beta$ -globin locus conferring integration site-independent gene expression

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THE regulatory elements that determine the expression pattern of a number of eukaryotic genes expressed specifically in certain tissues have been defined and studied in detail. In general, however, the expression conferred by these elements on genes reintroduced into the genomes of cell lines and transgenic animals has turned out to be at a low level relative to that of endogenous genes, and influenced by the chromosomal site of insertion of the exogenous construct. We have previously shown that if regions flanking the human  $\beta$ -globin locus are introduced into the mouse genome along with the human  $\beta$ -globin gene, a level of expression comparable to that of endogenous genes can be achieved that is also independent of integration site<sup>1,2</sup>. We have now defined a dominant control region with these properties consisting of 6.5 kilobases of DNA encompassing erythroid cell-specific DNase I hypersensitive sites. The identification of such dominant control regions could have important applications in somatic gene therapy.

The human  $\beta$ -globin 'minilocus' construct contained 21 kilobases (kb) of DNA from the region 5 to the  $\epsilon$ -globin gene encompassing four erythroid cell-specific DNase I hypersensitive sites and 12 kb of DNA 3' to the human  $\beta$ -globin locus<sup>1</sup>. To investigate the properties of these dominant control region (DCR) sequences, the 33 kb 5' and 3' sequences were replaced by a 6.5 kb DNA fragment containing only the upstream hypersensitive sites<sup>1,3,4</sup> (Fig. 1). Construct 1359 contains the four hypersensitive sites in the same orientation relative to the  $\beta$ -globin gene (and the *tk-neo*<sup>r</sup> gene; tk, thymidine kinase; neo<sup>r</sup>, neomycin-resistance) as that found on chromosome 11. Construct 1400 has the DNase I hypersensitive sites in the opposite orientation and 1401 and 1357 have the four DNase I hypersensitive sites placed 3' to the  $\beta$ -globin gene (Fig. 1).

The insert of construct 1359 was injected into fertilized mouse eggs and 56 embryos were collected after 13.5 days of gestation. Thirteen transgenics were obtained in two groups as defined by S1 nuclease protection (Fig. 2a) and Southern blot analysis to measure the expression levels and copy number of the human  $\beta$ -globin gene in the fetal liver (Table 1). Members of the first group expressed the insert at low levels because they were mosaics (three mice, for example lane 2), or carried a deletion in the insert (two mice, for example lane 3). The remainder express the gene at high levels in a copy-number dependent way: five of these looked normal (for example lane 4) and three looked anaemic (for example lane 1). These results are therefore similar to those obtained with the minilocus<sup>1</sup> and show that full erythroid-specific activation is obtained by the small reconstructed DCR, sometimes leading to a thalassaemia-like anaemia.

To study the different  $\beta$ -globin constructs and avoid mosaicism, three independent, stably transformed, MEL cell popula-



tions were generated for each construct<sup>1</sup>. RNA was prepared before (-) or after (+) differentiation<sup>5</sup> and analysed on northern blots with probes of similar specific activity. Figure 2b shows that human  $\beta$ -globin mRNA is present at levels as high as those of endogenous mouse globin mRNA after induction. For comparison, Fig. 2b shows equal amounts of RNA from two populations using a construct (1273) without the DCR. Human  $\beta$ -globin expression is increased at least 100-fold by the addition of the four DNaseI hypersensitive sites and this high level of expression is observed in all orientations and relative positions (1359, 1400, 1401 and 1357). Moreover, this level of expression seems to be higher than that seen previously with the  $\beta$ -globin minilocus<sup>2</sup> (1016, Fig. 2b). We used S1 nuclease analysis to quantitate the levels of mRNA using probes for the human  $\beta$ , mouse  $\alpha$  and mouse  $\beta^{\text{maj}}$  globin genes (Fig. 3a and Table 1). Hybridization signals from Southern blots to probes for *tk-neo*<sup>r</sup> and human  $\beta$ -globin genes were compared to those from the endogenous single-copy mouse *Thy-1* gene and average  $\beta$ -globin gene copy numbers were calculated (Fig. 3b, Table 1). The expression per gene copy ratio is 120% per human  $\beta$ -globin gene compared to the endogenous mouse  $\beta^{\text{maj}}$ -globin gene (see Table 1 legend). As observed in the embryos, the expression levels seem to be directly proportional to the gene copy number

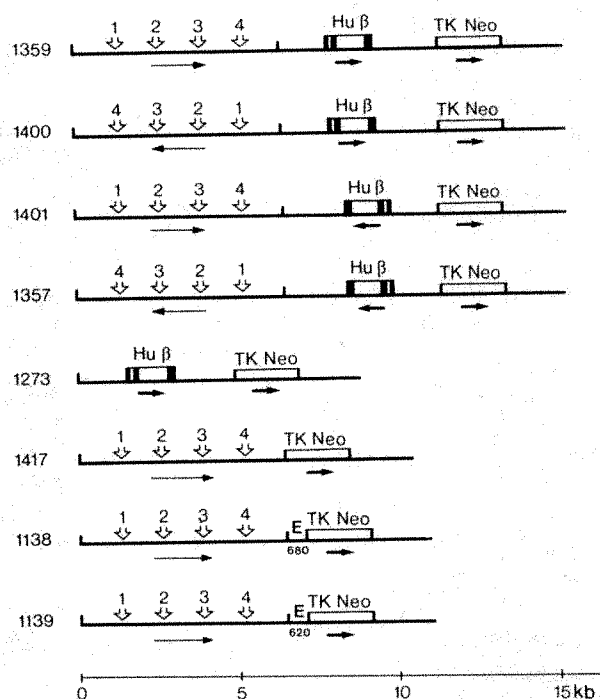


FIG. 1 Construction of the human  $\beta$ -globin plasmid locus.

**METHODS.** Plasmids 1359, 1400, 1401 and 1357 were constructed using restriction endonuclease fragments encompassing the 5' DNaseI hypersensitive sites ligated into a pPoly-III-I vector (a gift from A. J. Clark, Edinburgh) in the same orientation as in the normal  $\beta$ -globin locus: 2.1 kb *Bam*HI-*Xba*I fragment containing hypersensitive site 1, a 1.9 kb *Hind*III fragment containing hypersensitive site 2, a 1.5 kb *Asp*718-*Bgl*II fragment containing hypersensitive site 3 and a 1.0 kb partial *Sst*I-*Hind*III fragment containing hypersensitive site 4. A 6.5 kb *Not*I fragment containing all of the restriction fragments of the hypersensitive sites was then cloned into a Bluescript vector (1273) containing the 4.8 kb *Bgl*II fragment of the human  $\beta$ -globin gene<sup>1</sup> and a 2.7 kb partial *Eco*RI *tk-neo*<sup>r</sup> gene fragment from the cosmid pTCF (ref. 15). Vertical arrows show arrangement of the hypersensitive sites in the dominant control regions but do not show their actual positions within these constructs. Construct 1417 contains the 6.5 kb *Not*I fragment containing the four 5' hypersensitive sites cloned into a specially designed polylinker in a pUC18 vector containing the 2.0 kb partial *Nar*I-*tk-neo*<sup>r</sup> gene fragment. Construct 1138 contains the 680 bp *Dra*I-*Acl*I fragment of the 3' human  $\beta$ -globin enhancer cloned between the *Kpn*I and *Cla*I sites of 1417. Construct 1139 contains the 620 bp *Dra*I fragment of the internal enhancer of the human  $\beta$ -globin gene<sup>9</sup>. Construct 1016 is the  $\beta$ -globin minilocus cosmid<sup>1,2</sup>.

as was observed for the human  $\beta$ -globin minilocus<sup>1,2</sup>, although this argument is weakened by the fact that all the MEL populations generated have a similar average gene copy number (Fig. 3b, Table 1).

Figure 2b also shows that the 6.5 kb DNA fragment activates the heterologous herpes simplex virus (HSV) *tk* promoter in a similar way (compare 1359 to 1273). Unlike  $\beta$ -globin expression however, *neo*<sup>r</sup> transcripts are present at about the same level as with the  $\beta$ -minilocus cosmid (ref. 2 and 1016), possibly because the *tk-neo*<sup>r</sup> in the minilocus may already be transcribed maximally. Another difference between the minilocus and plasmid constructs is the higher level of  $\beta$ -globin and the *tk-neo*<sup>r</sup> transcripts before erythroid induction (Fig. 2b and ref. 2). This may be due to the loss of specific sequences or to the greatly reduced size of the constructs. Therefore, the reconstructed DCR does not isolate the  $\beta$ -globin gene from the effects of flanking sequences before differentiation.

To investigate further the activation of the heterologous HSV *tk* promoter, constructs were prepared which carried the 6.5 kb DCR without any human  $\beta$ -globin gene sequence (1417) or carrying the previously described enhancers, either from within (1139) or from the 3' flanking region of (1138) the  $\beta$ -globin gene<sup>6,7,8,9</sup>. Activation and erythroid inducibility of the HSV *tk* promoter is not dependent on the promoter or enhancers of the  $\beta$ -globin gene (Fig. 2c).

Interestingly the human  $\beta$ -globin gene expression per gene copy is increased, when compared to the  $\beta$ -globin minilocus (1016, Fig. 2b), but the *tk-neo*<sup>r</sup> expression remains the same. The transcription machinery of the *tk* promoter may have been

TABLE 1 Copy number and expression levels of the human  $\beta$ -globin gene in transgenic mice and transfected cells

		c.p.m. Hu $\beta$	Copy number Hu $\beta$ -globin	Percentage expression		
				Hu $\beta$ / Ma	Hu $\beta$ / Mb <sup>maj</sup>	Ma/ Mb <sup>maj</sup>
Mouse 1		315*	4.0	ND	160	ND
	2	449	mosaic	ND	—	ND
	3	654	deletion	ND	—	ND
	4	5,385	3.0	ND	160	ND
1357	a	14,738	7.0	70	130	190
	b	10,859	4.3	90	130	140
	c	10,692	2.6	110	210	200
1359	a	12,692	7.7	70	110	170
	b	18,855	7.0	100	140	150
	c	17,533	7.0	80	150	200
1400	a	19,670	5.8	90	100	100
	b	20,924	5.8	90	90	100
	c	18,695	6.2	90	90	100
1401	a	14,643	3.4	120	120	100
	b	17,835	4.5	100	110	110
	c	13,262	4.9	70	70	100
Average expression/gene copy				90	120	140
Standard deviation				16	36	42
1359	a	39,709	7.7	60	120	180
	3x b	55,012	7.0	90	150	160
	c	56,059	7.0	80	160	210
1016	a	2,684	5.0	18	50	270
	h	1,289	7.0	11	30	280

Copy numbers of human  $\beta$ -globin (Hu  $\beta$ ) were determined by Southern blotting and laser densitometry as described. Background-corrected Cerenkov counts for human  $\beta$ -globin, mouse  $\beta$ -globin major and mouse  $\alpha$ -globin protected fragments were expressed as ratios of the exogenous human  $\beta$ -globin message (Hu  $\beta$ ) to the endogenous mouse  $\alpha$  and  $\beta^{\text{maj}}$  signals (Ma and Mb<sup>maj</sup>), correcting for relative specific activities. This expression ratio was then reduced to an expression per gene copy ratio by dividing it by the ratio of the number of copies of human  $\beta$ -globin to the number of endogenous gene copies (two for mouse  $\beta$ -globin major and four for mouse  $\alpha$ -globin). Clones a and h seem to have threefold lower human  $\beta$ -globin expression than the average minilocus MEL population due to the loss of endogenous mouse  $\beta$ -globin genes<sup>2</sup>. ND, not determined, \* Anaemic.

saturated, which also explains why the addition of human  $\beta$ -globin gene enhancers to the 6.5 kb DNA fragment does not result in any further increase in the number of *tk-neo*<sup>r</sup> transcripts in MEL cell populations (Fig. 2c). We therefore suggest that a requirement for reproducible but low expression levels of biologically active molecules could be met by the combination of the DCR and inefficient or mutagenized promoters.

DNaseI fadeout analysis of nuclei from uninduced cells

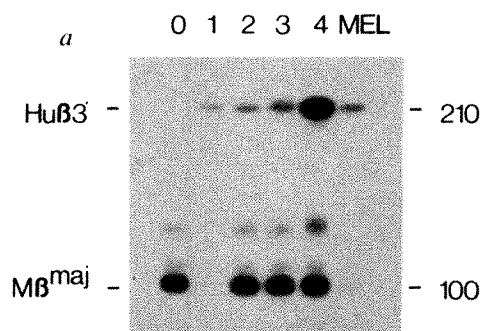
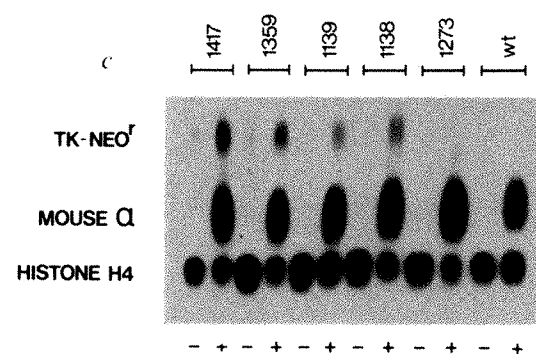
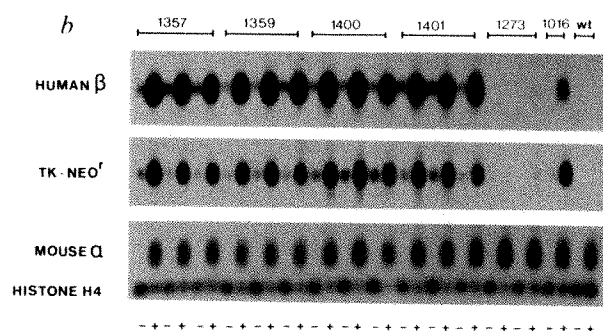


FIG. 2 Analysis of human  $\beta$ -globin expression. *a*, S1 nuclease protection analysis of RNA from the transgenics (sizes are shown on the right). *b*, Northern blot of RNA prepared before (–) or after (+) differentiation of the stably transformed MEL cell lines. Lanes marked 1016 contain RNA from a MEL cell clone stably transfected with the human  $\beta$ -globin minilocus<sup>2</sup>. Wt, RNA from untransfected MEL C88 cells. The probes were for human  $\beta$ -globin (760 bp *EcoRI*–*MspI*) or the *tk-neo*<sup>r</sup> gene (580 bp *SphI*–*BglII*). The filter initially probed with human  $\beta$ -globin was reprobed with both the mouse histone H4 probe (460 bp *EcoRI*–*Tth111I*) and the mouse  $\alpha$ -globin probe (300 bp *BamHI*). *c*, Northern blot of total RNA (10  $\mu$ g) from uninduced (–) and induced (+) transfected C88 cell populations, probed simultaneously with the *tk-neo*<sup>r</sup> probe, the mouse  $\alpha$ -globin probe and the mouse histone H4 probe.

**METHODS.** In (*a*), an 11.5 kb *EcoRV* fragment from construct 1359 was gel-purified and microinjected into fertilized mouse eggs<sup>1</sup>. The levels of human  $\beta$ -globin mRNA were determined by S1 nuclease analysis with a mixture of probes for the human  $\beta$ -globin and mouse  $\beta$ <sup>maj</sup>-globin genes<sup>1,2</sup>. After autoradiography, each band was excised from the gel, placed at the bottom of an Eppendorf tube and quantitated by Cerenkov counting. A local background count was obtained by measuring a gel slice immediately above the band of interest and was subtracted from the actual count in subsequent calculations (see Table 1). For the Northern blots (*b*, *c*), plasmid DNA of indicated constructs (100  $\mu$ g) was linearized by digestion with *PvuI* and introduced into MEL C88 cells by electroporation<sup>9</sup>. Three independent G418-

shows that the hypersensitive sites 1–4 are regenerated in uninduced MEL cells, but not in L-cells with the exception of site 3 (data not shown and ref. 2). Weaker DNaseI hypersensitive sites can be seen on the  $\beta$ -globin gene promoter, 3' enhancer and *tk-neo*<sup>r</sup> promoter. Therefore, at this level of resolution, the establishment of an 'active' chromatin configuration is separable from full  $\beta$ -globin gene transcription.

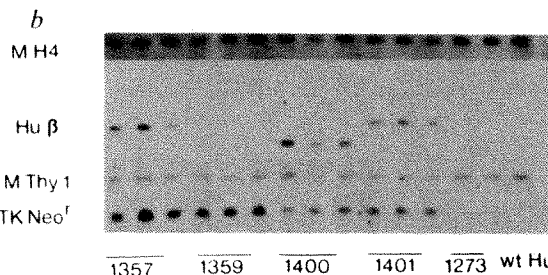
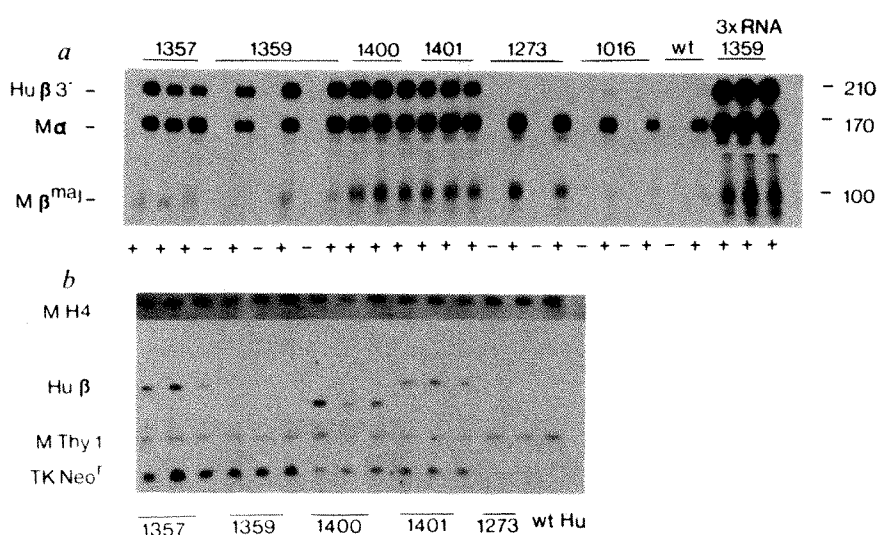
The human  $\beta$ -globin gene constructs in these experiments are



resistant populations of 50–100 clones were generated for each construct. Total RNA (10  $\mu$ g) isolated by LiCl/urea extraction<sup>16</sup> from uninduced MEL cells, and MEL cells induced to differentiate by the addition of 2% v/v dimethyl sulphoxide (+) for five days, was electrophoresed on duplicate 1.5% agarose denaturing gels containing 0.7% formaldehyde and ethidium bromide (1  $\mu$ g ml<sup>–1</sup>) blotted to nitrocellulose and probed.

FIG. 3 S1 nuclease analysis and copy number determinations. *a*, S1 nuclease analysis of total RNA from uninduced (–) and induced (+) MEL cells. *b*, Southern blotting of genomic DNA from MEL populations.

**METHODS.** (*a*) Total RNA (10  $\mu$ g) from uninduced (–) and induced (+) populations of MEL cells were hybridized to a mixture of mouse  $\beta$ -globin major, mouse  $\alpha$ -globin and human  $\beta$ -globin probes described previously<sup>1,2</sup>. Relative specific activities were 1, 10 and 8, respectively, for the above probes. Three controls containing 30  $\mu$ g of RNA (3  $\times$  RNA) were performed to prove that the S1 probes were in excess. Quantitation was as described in Fig. 2a. 1016 RNAs are from two MEL cell clones containing the human  $\beta$ -globin minilocus<sup>2</sup>. Sizes are shown on the right. *b*, Genomic DNA (8  $\mu$ g) of MEL populations was digested with *EcoRI* and electrophoresed on a 0.6% agarose gel. After Southern blotting, the filter was probed with human  $\beta$ -globin intervening sequence probe (900 bp *BamHI*–*EcoRI*) and subsequently with a *tk-neo*<sup>r</sup> resistance probe (580 bp *SphI*–*BglII*), a mouse Thy-1 probe (M Thy 1) (600 bp *PstI*) and a histone H4 probe (MH4) (460 bp *EcoRI*–*Tth111I*). Laser densitometry using a range of autoradiographic exposures was used to quantitate human  $\beta$ -globin copy number. Mouse Thy-1 and histone H4 were used to correct for DNA loading. Human placental DNA was used to obtain an estimate of the actual  $\beta$ -globin copy



number. The human  $\beta$ -globin copy number of the populations of cells containing 1359 were compared to the other constructs through the *tk-neo*<sup>r</sup> gene signal as the *EcoRI* digestion gave a larger 9.3 kb human  $\beta$ -globin *EcoRI* fragment.

integrated at different sites into host cell DNA and have been replicated as chromatin. This contrasts with transient transfections using extra-chromosomal DNA templates not packaged into chromatin. Viral enhancers can stimulate  $\beta$ -globin gene expression several hundredfold in transient assays<sup>10</sup>, but this is not observed after stable integration, and levels of expression are dependent upon the site of integration into host DNA.

The observation that only 6.5 kb of DNA from the 5' boundary of the human  $\beta$ -globin locus allows high levels of expression of the human  $\beta$ -globin gene and the heterologous *tk-neo*<sup>r</sup> gene in erythroid cells has implications for the prospects of somatic gene therapy. Currently, the most realistic approach for therapy of a  $\beta$ -globin gene disorder (thalassaemia or sickle-cell anaemia) is by *in vitro* retroviral infection of bone marrow and transplantation<sup>11-14</sup>. Retroviruses carrying the human  $\beta$ -globin gene give rise to low levels of expression that are dependent upon the site of integration. The observation that the DCR works equally efficiently in all orientations and positions should facilitate production of retrovirus stocks with higher titres. The size of the reconstituted DCR with a smaller human  $\beta$ -globin gene

is within the range that can be packaged into retroviral particles. Such constructs have been made and are currently being tested. □

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## Identification of globular mechanochemical heads of kinesin

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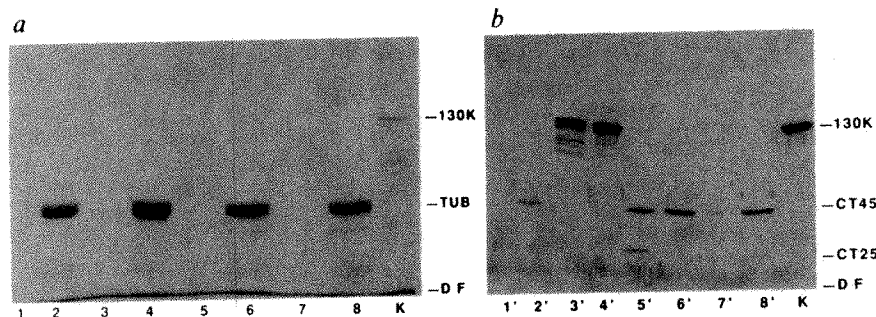
KINESIN is a mechanoenzyme which uses energy liberated from ATP hydrolysis to transport particles towards the 'plus ends' of microtubules<sup>1-6</sup>. The enzyme consists of two polypeptide heavy chains of relative molecular mass ( $M_r$ )  $\approx$  110,000-140,000 (110K-140K) plus copurifying light chains; these polypeptides are arranged in a structure consisting of two globular heads attached to a fibrous stalk which terminates in a 'feathered' tail<sup>7-11</sup>. Here we report that a function-disrupting monoclonal antikinesin, which binds to the 45K fragment of the kinesin heavy chain<sup>12,13</sup>, recognizes an epitope located towards the N-terminal end of the heavy chain, and decorates the two globular heads lying at one end of the intact molecules (one antibody per head). The results show

that the two heavy chains of native kinesin are arranged in parallel, and that the 45K fragments, which display nucleotide-sensitive interactions with microtubules<sup>12,13</sup>, represent mechanochemical 'heads' located at the N-terminal regions of the heavy chains. Thus, it is likely that the kinesin heads are analogous to the subfragment-1 domains of myosin.

Electron microscopy<sup>7,10,11</sup> and DNA sequence analysis<sup>15</sup> indicate that kinesin heavy chains are organized into distinct domains. We have obtained evidence for such domains from the proteolytic cleavage of kinesin, which yields heavy-chain fragments of  $M_r \approx$  45K and 76K<sup>12,13</sup>. The 45K fragment is bound by three monoclonal antikinesins that inhibit kinesin-driven microtubule motility<sup>12</sup>, one of which, SUK 4, was used in the present study. Interestingly, the proteolysis reaction that produces the SUK 4-reactive 45K fragments is nucleotide- and microtubule-sensitive (Fig. 1). Irrespective of the presence or absence of microtubules, the 130K kinesin heavy chain is resistant to cleavage in the absence of nucleotide whereas the formation of the 45K peptide is enhanced by magnesium ATP. In the absence of microtubules, MgATP also promotes the formation of a small quantity of a 25K subfragment of the 45K fragment (Fig. 1, lane 5). The presence of AMPPNP (an unhydrolysable ATP analogue) or ADP seems to favour subdigestion of the 45K domain in the absence of microtubules; consequently no SUK 4-reactive products are visible on immunoblots. The 45K fragment is, however, stabilized by the presence of microtubules in AMPPNP or ADP. It seems therefore that the proteolytic reactions that produce and subdigest the 45K fragment are affected by the conformational differences of kinesin-micro-

FIG. 1 Nucleotide and microtubule-sensitive proteolysis of sea-urchin kinesin 130K heavy chains. *a*, Coomassie-stained SDS polyacrylamide gel; *b*, corresponding immunoblot probed with the function-blocking monoclonal antikinesin SUK 4, which binds to the 45K peptide<sup>12</sup>.

METHODS. Partially purified sea-urchin kinesin (lanes marked k) was obtained by AMPPNP-microtubule affinity binding, MgATP release, and Biogel A5M chromatography in the absence of MgATP (ref. 12). The kinesin in our standard PMEG buffer<sup>5</sup> was digested with  $\alpha$ -chymotrypsin<sup>12</sup> in the presence (lanes 2, 4, 6, 8) or absence (lanes 1, 3, 5, 7) of microtubules (2 mg ml<sup>-1</sup>) assembled using taxol from purified bovine brain tubulin. Lanes 3 and 4 were incubated with apyrase 50  $\mu$ g ml<sup>-1</sup> to remove residual ATP or ADP; lanes 5 and 6 show digests performed in MgATP (5 mM), whereas 7 and 8 were performed in the presence of Mg AMPPNP (5 mM). Lanes 1 and 2 show digests performed using kinesin from the Biogel column and which presumably contained bound MgADP (ref.



4) (identical results were obtained in parallel digestion reactions performed in the presence of additional MgATP (1 mM) although in the presence of NaCl (0.5 M) microtubules did not protect the 45K peptide). 130K, intact kinesin heavy chain; TUB, tubulin; CT45, the 45K chymotryptic fragment of the kinesin heavy chain; CT25, a minor 25K fragment; DF, dye front.



tubule-nucleotide states and thus resemble the proteolysis of the myosin-head domain, a reaction which is affected by the molecular movements that occur as myosin interacts with nucleotides and actin<sup>16</sup>. We propose therefore that the 45K fragment is a mechanochemical domain on kinesin that contains nucleotide- and microtubule-binding sites<sup>12,13</sup>, and undergoes conformational changes as it interacts with microtubules and nucleotides to generate motile force.

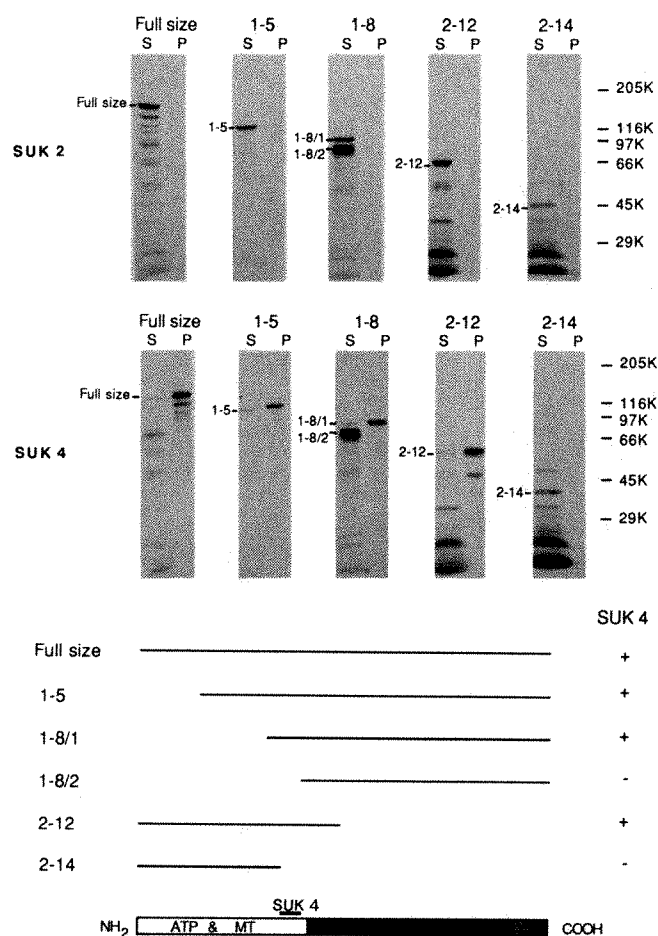


FIG. 2 Localization of the region of kinesin containing the SUK 4 epitope by immunoprecipitations of truncated *Drosophila* kinesin heavy-chain molecules. **a**, Upper panels, autoradiograms of the immunoprecipitation by SUK 2 and SUK 4. Each polypeptide is indicated by a line and its number in the corresponding autoradiogram. S, supernatant; P, pellet. **b**, Schematic representation of the kinesin heavy chain showing the position of the SUK 4 epitope, and the globular N-terminal domain (open box) and the nucleotide (ATP) and microtubule (MT) binding regions defined by previous studies<sup>15</sup>. Translation products that reacted with SUK 4, +. Black region, the  $\alpha$ -helical coiled-coil domain, which ends in a second globular domain (shaded) at the terminus. In this model, SUK 4 lies in residues 320–390 and is near a microtubule binding region.

**METHODS.** Deletions were introduced into the full-length *Drosophila* kinesin heavy-chain cDNA, cloned into pBluescript plasmids (Stratagene) as described previously<sup>14,15</sup> and the truncated products were transcribed, and translated using a reticulocyte lysate system<sup>14,15</sup>. Six translation products were analysed: the full size *Drosophila* kinesin heavy-chain polypeptide consisting of 975 residues; 1-5, which lacks the first 130–170 N-terminal residues; 1-8/1, which lacks ~320 N-terminal residues; 1-8/2, which arises as a result of an internal initiation even in the same cDNA that produces 1-8/1 (ref. 15), and lacks ~390 N-terminal residues; 2-12, which lacks 500 C-terminal residues and 2-14 which lacks 640 C-terminal residues. The lysates containing the expressed, truncated products were incubated with monoclonal antikeratin, SUK 4, which cross-reacts with *Drosophila* kinesin heavy chains<sup>12</sup>, and (as a control) with SUK 2, which reacts with sea urchin but not *Drosophila* kinesin heavy chains<sup>12</sup>. The antibody-antigen complexes were immunoprecipitated using pansorbin *Staphylococcus aureus* cells, and subjected to SDS gel electrophoresis and autoradiography to locate the precipitated polypeptides.

To locate the 45K fragment on the kinesin heavy chain, we first determined the position of the SUK 4 epitope in the protein sequence (Fig. 2). Kinesin heavy-chain molecules carrying deletions of various lengths were produced by expressing truncated versions of the *Drosophila* kinesin heavy-chain gene<sup>14,15</sup>. The binding of SUK 4 to the truncated protein products was assayed by immunoprecipitation: SUK 2, an IgG1 that does not bind to *Drosophila* kinesin<sup>12</sup>, was used as a negative control.

Six translation products were analysed (described fully in ref. 15 and briefly in Fig. 2). As expected, SUK 2 did not immunoprecipitate any of the translation products. The full-size polypeptide (975 residues) and the truncated proteins 1-5, 1-8/1 and 2-12 but not 1-8/2 or 2-14, however, were immunoprecipitated by SUK 4. Significantly, molecules carrying deletions that include residues ~320–390 were not immunoprecipitated by SUK 4. These results suggest therefore that the epitope recognized by SUK 4 lies within the N-terminal third of the kinesin heavy chain, and is probably within residues 320–390. Strikingly, previous studies by Yang *et al.*<sup>15</sup> show that the truncated kinesin heavy chain, 1-8/1, binds to microtubules weakly in sedimentation assays, whereas 1-8/2 does not bind microtubules at all. This indicates that the SUK 4 epitope is located near a region of the kinesin heavy chain that interacts with microtubules.

Yang *et al.*<sup>15</sup> determined the sequence of the *Drosophila* kinesin heavy chain, and predicted that it consists of three structural domains: a globular N-terminal domain; a central  $\alpha$ -helical coiled coil region; and another globular domain at the C-terminal region. In their proposed model, the N-terminal globular domain contains nucleotide- and microtubule-binding sites<sup>15</sup> as well as the SUK 4 epitope (Fig. 2). The SUK 4 epitope and the microtubule- and nucleotide-binding sites are also all localized on the 45K fragment of kinesin, indicating that this 45K peptide corresponds to the globular N-terminal domain predicted from the sequence analysis.

To test this interpretation, we used immunoelectron microscopy to visualize the region of kinesin containing the SUK 4 epitope. The lower panels of Fig. 3a show freeze-etch electron micrographs of individual kinesin molecules as elongated bipolar structures approximately 75 nm in length. Each has two small, symmetrical globular 'heads' (9–10 nm in diameter) at one end of a long stalk (3–5 nm in width), which terminates in an irregular, bipartite 'feathered tail'. The appearance of this tail varies among molecules more than any other ultrastructural feature. One interpretation of these images is shown in Fig. 3b. The upper panels in Fig. 3a show electron micrographs of kinesin molecules decorated with SUK 4. This antibody clearly binds to the globular heads of the molecule, resulting in a marked increase in mass. Typically, this mass is the size of two IgG molecules (diameter 30 nm) and consists of at least six smaller lobes. Because IgG molecules themselves have a tri-lobed appearance<sup>17</sup>, we conclude that each of the two heads binds its own SUK 4 antibody.

Our results indicate that the N-terminal regions of two heavy chains form globular heads on the kinesin molecule (Fig. 3b). We propose that these heads are 'motor' domains undergoing changes in conformation as they interact with nucleotides and microtubules to generate force and motion, and that antibodies such as SUK 4 which bind to these heads can inhibit kinesin-driven motility by interfering with mechanochemical coupling. The junction between the head and the stalk may be flexible, rendering it highly susceptible to proteolysis, as in the case of myosin. In this model, the 45K fragments correspond to detached globular heads that are analogous to myosin subfragment 1(S1). The microtubule-induced ATPase activity of the 45K fragment, like the actin-induced ATPase activity of myosin S1 (ref. 18), is higher than that of the parent enzyme from which it is derived<sup>13</sup>, but whether the mechanism responsible for the elevation of ATPase activity is the same in these two cases remains to be determined.

Kinesin, myosin and dynein thus show striking similarities

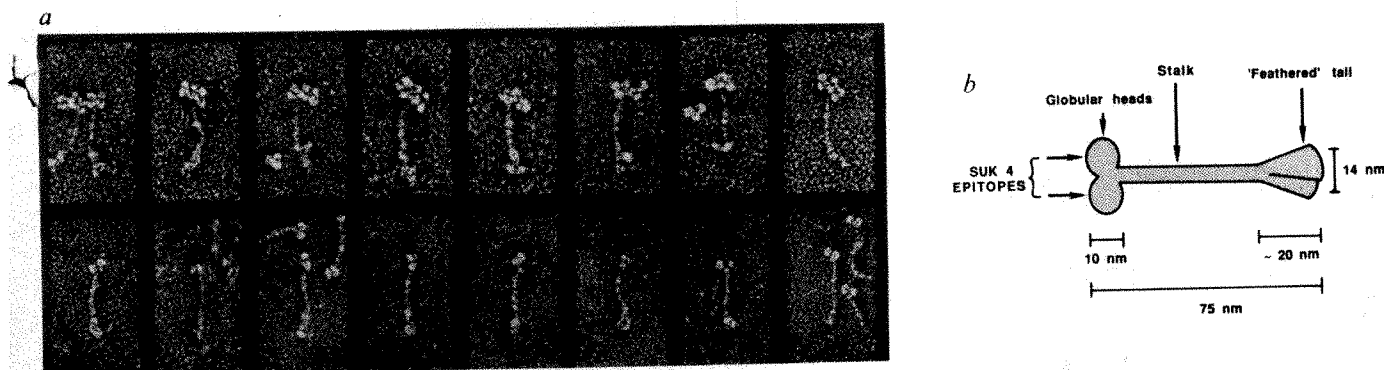


FIG. 3 a, Electron micrographs comparing individual sea-urchin kinesin molecules (lower row) with kinesin molecules reacted with the monoclonal antikinesin SUK 4 (upper row). The antibody binds to the two globular heads at the upper ends of the molecules. These represent rotary-shadowed platinum replicas of kinesin molecules (prepared as in Fig. 1 in PMEG buffer without nucleotide) that were adsorbed to microscopic flakes of mica and then freeze-dried by techniques described previously<sup>17</sup>. Magnification 144,000 $\times$ . b, Model of the ultrastructural morphology of individual kinesin molecules. The globular heads, corresponding to the N-terminal domains predicted from the amino-acid sequence<sup>15</sup>, contain the ATP and MT binding sites, and the SUK 4 epitopes. The stalk is thought to consist of an  $\alpha$ -helical coiled coil region (designated by the black area in Fig. 2), which terminates in a heterogeneous, bipartite feathered tail (the shaded C-terminal region in Fig. 2 is thought to interact with additional material to generate structures of the size seen in the micrographs<sup>15</sup>).

in their morphology, consisting of multiple globular mechanochemical heads attached to flexible stalks. The significance of this structural organization, however, is not clearly understood. It is possible that by having more than one head the mechanoenzyme remains attached as it tracks along its cytoskeletal filament with each head undergoing cycles of attachment and detachment. Single-headed myosin-1, however, also seems able to generate sliding between adjacent actin filaments<sup>20</sup>, and to transport beads and organelles along cytoskeletal filaments<sup>21,22</sup>. Johnson<sup>23</sup> has suggested that the flexible segment allows the production of a unidirectional force by mechanoenzymes whose motor domain attaches to its filament and undergoes equal and opposite structural changes during two different steps within the mechanochemical pathway. □

**Note added in proof:** Hirokawa *et al.*<sup>24</sup> have independently arrived at the conclusion that the two small heads on the kinesin molecule bind to microtubules.

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## High-resolution (1.5 Å) crystal structure of phospholipase C from *Bacillus cereus*

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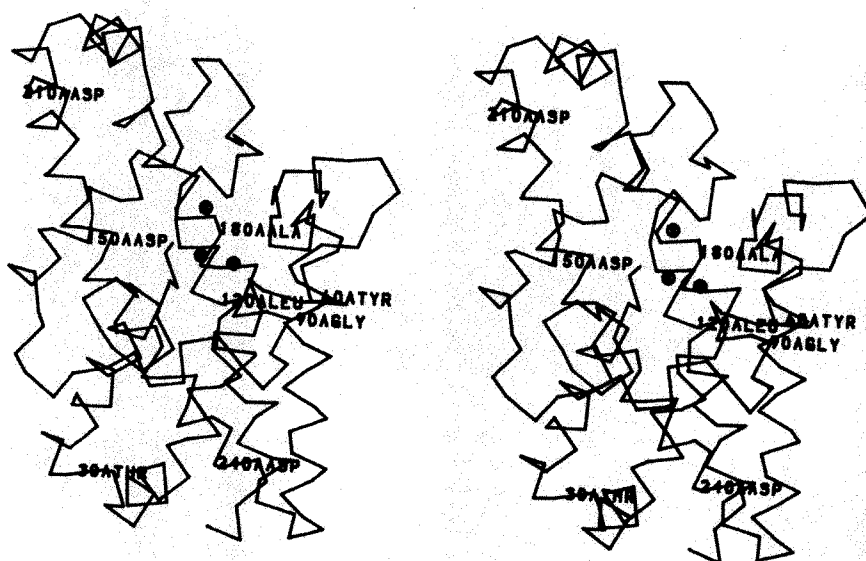
**BOTH** the phosphatidylinositol-hydrolysing and the phosphatidylcholine-hydrolysing phospholipases C have been implicated in the generation of second messengers in mammalian cells<sup>1,2</sup>. The phosphatidylcholine-hydrolysing phospholipase C (PLC) from *Bacillus cereus*, a monomeric protein containing 245 amino-acid residues<sup>3</sup>, is similar to some of the corresponding mammalian proteins<sup>4</sup>. This, together with the fact that the bacterial enzyme can mimic the action of mammalian PLC in causing, for example, enhanced prostaglandin biosynthesis<sup>5</sup>, suggests that *B. cereus* PLC can be used as a model for the hitherto poorly characterized mammalian PLCs. We report here the three-dimensional structure of *B. cereus* PLC at 1.5 Å resolution. The enzyme is an all-helix protein belonging to a novel structural class and contains, at least in the crystalline state, three Zn<sup>2+</sup> in the active site. We also present preliminary results from a study at 1.9 Å resolution of the complex between PLC and inorganic phosphate (P<sub>i</sub>) which indicate that the substrate binds directly to the metal ions.

The crystal structure of PLC has been solved at 2.8 Å resolution using multiple isomorphous replacement and solvent flattening<sup>6</sup>, and refined to 1.5 Å through the intermediate resolutions at 2.5, 2.3 and 1.9 Å. The present *R*-factor, using all the data and including 235 water molecules, is 15.7%. The molecular structure is shown in Figs 1 and 2. The crystallographic work, summarized in Table 1, will be published in more detail elsewhere.

The most striking feature of the molecule is the long anti-parallel helix pair formed by helix H and helices F and G, which can be compared with those of citrate synthase<sup>6</sup> and a short helix pair in phospholipases A<sub>2</sub> (ref. 7). Proline 218 in helix H induces a bend between helices H<sub>1</sub> and H<sub>2</sub>. Helix E lies across both this bend and the loop between helices F and G, giving the impression that the long helices are folded round it. The bend in helix B at Trp 43 is probably associated with its packing against the body of the molecule.

‡ Deceased.

FIG. 1 Stereo drawing of the backbone of PLC. Metal ions are shown as filled circles.



Helices A, B, C, D, F and H<sub>2</sub> form a compact arrangement of mixed parallel and antiparallel helices with a predominantly right-handed twist. Helix D runs diagonally within the helix assembly to lie behind the active site at its C-terminal end (Fig. 2). Although PLC seems to be the first enzyme with an all-helix conformation (citrate synthase contains a 13-residue  $\beta$ -sheet segment<sup>41</sup>), several enzymes contain tightly packed helix domains, although there seems to be no common topology (Fig. 2b, I-V).

The N-terminal tryptophan is buried immediately behind the active site and is involved in metal binding (Fig. 3). PLC is probably synthesized as a pro-enzyme with a 14-residue N-terminal extension<sup>3</sup> which could block the active site cleft to render the enzyme inactive and could also alter the metal binding. The last two residues are of the C terminus, which lies at the end of helix H<sub>2</sub>, disordered on the surface of the molecule. All non-helical stretches form external, 'random coil' loops between the helices (Fig. 2).

The amino-acid sequence of PLC shows no significant similarity to those of phospholipases A<sub>2</sub> (ref. 7). There is, however, some structural similarity with bovine<sup>8</sup>, porcine<sup>9</sup> and rattlesnake phospholipase A<sub>2</sub> (ref. 10). Superimposition of the antiparallel (residues 39–58 and 89–108) helix pairs in the latter over helices F and H<sub>2</sub> in PLC results in an approximate overlap of the N-terminal helices in phospholipase A<sub>2</sub> over helix A in PLC and a similar location for the N-terminal residue. In this orientation, the  $\beta$ -sheet region of phospholipase A<sub>2</sub> lies over the E-F loop in PLC. The active sites in the two enzymes, however, are well separated when the molecules are thus superimposed.

PLC is inhibited by P<sub>i</sub> and by monovalent anions<sup>11</sup>, including I<sup>-</sup>. The latter ion blocks the entrance to a cleft 5 Å wide, 8 Å deep and bounded on its external surface by the N-terminal loop, the helix B<sub>2</sub>-helix C loop, the helix D-helix E loop and the N-terminal end of helix E. The essential metal ions<sup>12</sup> lie at the bottom of this cleft and are liganded to residues from these loops and from helices A, B<sub>2</sub>, D and E, thus crosslinking several disparate parts of the protein chain (Fig. 3b). This probably explains the high conformational stability of the holoenzyme relative to the apoenzyme<sup>13</sup>. Furthermore, a 1.9 Å study of P<sub>i</sub>-PLC, now approaching completion (*R*-factor = 19%), shows that P<sub>i</sub> is closely associated to all three metal ions, with its oxygen atoms replacing two of the coordinated water molecules (Fig. 3c). This confirms the identification of the active site and indicates that the catalytic function of the metal ions is similar to that of the metal ions in alkaline phosphatase from *Escherichia coli*<sup>14</sup>, DNA polymerase I (refs 15 and 16) and bovine pancreatic deoxyribonuclease I (ref. 17). There are two distinct groups of residues in the active site: Glu 4, Asp 55, Tyr 56 and Glu 146

form an acidic pocket at one end, whereas Ser 64, Thr 65, Phe 66, Phe 70, Ile 80, Thr 133, Asn 134, Leu 135 and Ser 143 line the remainder. A similar differentiation of active-site residues is discernible in the phospholipases (ref. 18).

Metal bonding and exchange studies<sup>12,19</sup> have indicated the presence of two tightly bound Zn<sup>2+</sup> in active PLC. This was apparently confirmed during the search for heavy-atom derivatives by our identification of two sites where Zn<sup>2+</sup> was replaced with Cd<sup>2+</sup> (ref. 11). The interpretation of electron density maps in what has now proved to be the N-terminal loop region, however, has always been complicated by the presence of high electron density, similar to that of the other two metal ions, which was closely associated with Trp 1, His 14 and Asp 122, and which could not be explained by amino-acid structure. Contact

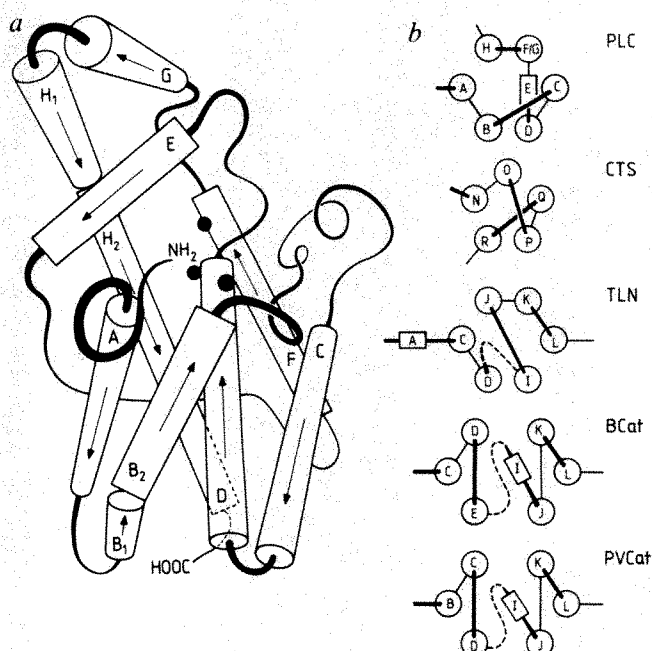


FIG. 2 a, Schematic drawing of the PLC fold with  $\alpha$ -helices shown as cylinders and metal ions as filled circles. Overall dimensions are 60 × 40 × 30 Å, 66% of residues fold as  $\alpha$ -helix and no  $\beta$ -sheet (predicted<sup>28</sup>, 30–36% helix, 24–30% sheet). Amino acids in helices: A, 12–28; B<sub>1</sub>, 33–44; B<sub>2</sub>, 44–55; C, 85–104; D, 105–125; E, 140–153; F, 171–187; G, 192–202; H<sub>1</sub>, 206–216; and H<sub>2</sub>, 216–243. b, Topology of PLC compared with helix domains in CTS (small domain)<sup>6</sup>, thermolysin (TLN)<sup>29</sup>, beef catalase (BCat)<sup>30</sup> and *Penicillium vitale* catalase (PVCat)<sup>31</sup>. Figures constructed after Levitt and Chothia<sup>32</sup>.



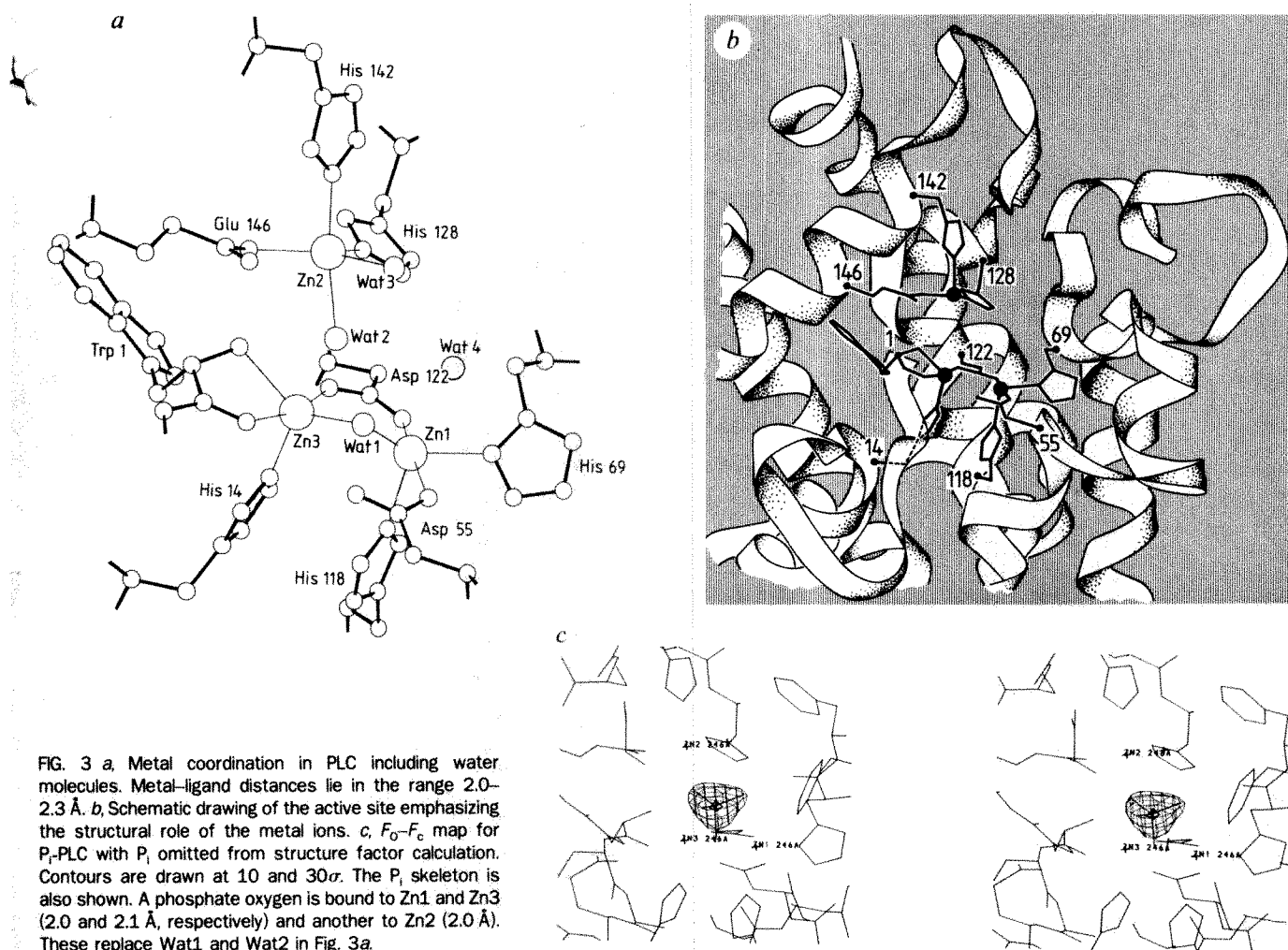


FIG. 3 *a*, Metal coordination in PLC including water molecules. Metal-ligand distances lie in the range 2.0–2.3 Å. *b*, Schematic drawing of the active site emphasizing the structural role of the metal ions. *c*,  $F_0 - F_c$  map for  $P_1$ -PLC with  $P_i$  omitted from structure factor calculation. Contours are drawn at 10 and 30 $\sigma$ . The  $P_i$  skeleton is also shown. A phosphate oxygen is bound to Zn1 and Zn3 (2.0 and 2.1 Å, respectively) and another to Zn2 (2.0 Å). These replace Wat1 and Wat2 in Fig. 3*a*.

TABLE 1 Summary of crystallographic data

Derivative	$N_h$		Resolution limit (Å)							
			14.1	6.45	5.00	4.20	3.65	3.37	3.10	2.80
$Cd^{2+} *$	2	$F$	0.68	0.79	0.72	0.67	0.87	0.79	0.75	0.85
		$R_c$	0.79	0.64	0.75	0.69	0.75	0.76	0.76	0.70
$Cd^{2+} \dagger$	2	$F$	1.31	0.96	0.90	0.70	0.64	0.51	0.55	—
		$R_c$	0.57	0.70	0.61	0.70	0.69	0.73	0.64	—
$PtCl_6^{2-} *$	1	$F$	0.43	0.61	0.60	0.55	—	—	—	—
		$R_c$	0.69	0.69	0.71	0.75	—	—	—	—
$PtCl_4^{2-} * \ddagger$	3	$F$	1.12	1.08	1.03	0.84	0.72	0.74	0.88	0.84
		$R_c$	0.65	0.67	0.76	0.74	0.70	0.78	0.68	0.76
$I^*$	6	$F$	1.42	1.32	1.17	0.97	0.76	0.81	1.02	1.10
		$R_c$	0.57	0.56	0.56	0.55	0.52	0.53	0.55	0.63
No reflections			406	677	846	989	1,091	1,220	1,314	1,137
Mean figure of merit			0.95	0.92	0.92	0.90	0.84	0.80	0.71	0.58

Crystallization: vapour phase diffusion; 5 mg ml<sup>-1</sup> PLC in 35% saturated ammonium sulphate; crystals up to 1.5 mm obtained in 3–4 weeks<sup>33</sup>.

Space group:  $P4_32_12$ ;  $a = 89.93(3)$  Å,  $c = 73.99(4)$  Å; solvent content, 53%;  $M_r = 28,585$ ; 1 molecule per asymmetric unit; data collected on Enraf-Nonius CAD4 diffractometer; native data (1.5 Å) collected on Station 9.6 at Daresbury, U.K.<sup>34</sup>,  $\lambda = 0.88$  Å, 51 film packs, 235,379 reflections merged to 46,061 unique data (99.2% of possible),  $R_{sym}$  for  $I_{hkl} = 0.056$ .

Space group ambiguity was resolved by calculation of SIRAS phases to 4 Å using the anomalous differences of the  $Cd^{2+}$  derivative measured with Cr-K $\alpha$  radiation in  $P4_12_12$  and  $P4_32_12$ ; only the latter gave sensible difference maps for the other derivatives.

Abbreviations:  $N_h$ , number of heavy atom sites;  $F$ , (r.m.s. heavy-atom structure factor)/(r.m.s. lack of closure);  $R_c$ , centric  $R$ -factor ( $\sum(|F_{PH} - F_P| - |F_H|)/\sum|F_{PH} - F_P|$ ) where  $F_{PH}$  and  $F_P$  are the structure factor amplitudes observed for derivative and native respectively, and  $F_H$  is the calculated heavy-atom structure factor amplitude).

Refinement: Konnert-Hendrickson with standard restraints<sup>35</sup>,  $R = 15.7\%$  at 1.5 Å for 45,743 reflections (1.9 Å diffractometer data merged with 1.5 Å synchrotron data) with no  $\sigma F/F$  cutoff and including 235 water molecules. Deviations (r.m.s.) from ideality are: bond distances, 0.021 Å; angle distances, 0.049 Å; planarity 0.014 Å. Luzatti plot<sup>36</sup> corresponds to r.m.s. coordinate error of 0.15 Å. Final refinement and the assignment of water molecules is not yet complete.

\* Graphite-monochromated Cu-K $\alpha$  radiation.

† Cr-K $\alpha$  radiation with vanadium  $\beta$ -filter.

‡ Group scattering factors<sup>37</sup> used for  $PtCl_4^{2-}$ .

distance and charge considerations lead us to conclude that this density is due to a third  $\text{Zn}^{2+}$  (no other metals have been detected in native PLC), which is liganded to the amino and carboxyl groups of Trp 1 to form a five-membered chelate ring similar to that observed in many Zn-amino acid complexes (for example, with aspartic<sup>20</sup> and glutamic<sup>21</sup> acids). The third  $\text{Zn}^{2+}$  is also coordinated to His 14 and Asp 122.

The resulting constellation of metal ions resembles that in alkaline phosphatase from *E. coli*<sup>14</sup>; one of a family of enzymes that have an absolute requirement for  $\text{Zn}^{2+}$  but can bind other metals with retention of activity<sup>22</sup>. The Zn-Zn distances in PLC are similar to those reported for Cd-substituted alkaline phosphatase<sup>14</sup>. Interestingly, the synthesis of both PLC and alkaline phosphatase are P<sub>i</sub>-repressed in *B. cereus* (P. M. Guddal, K. Schulstad, T. Johansen and C.L., unpublished observations) and together represent a P<sub>i</sub> retrieval system for the bacterium. The similarity in the active sites in the two enzymes may therefore be more than coincidental (alkaline phosphatase hydrolyses the product from PLC).

Figure 3a shows the environment of the metal ions. Asp 122 forms a carboxylate bridge between Zn1 and Zn3 (Zn-Zn distance 3.3 Å). Two such bridges occur in myohemerythrin (Fe-Fe, 3.23 Å) (ref. 23) and are probably formed by Asp 51 in AP (ref. 14) and between the metal ions in DNA polymerase I (ref. 16). A molecule of water or OH<sup>-</sup> forms a second bridge between Zn1 and Zn3; again this is similar to the oxide bridge in myohaemerythrin. The coordination of Zn2 resembles that in carboxypeptidase<sup>24</sup>, except that only one of the carboxyl oxygens from Glu 146 is bonded to the metal ion. Metal coordination is completed by two further water molecules so that all three metal ions are approximately trigonal bipyramidal. Histidines 14 and 118 are approximately parallel and are oriented in a manner which resembles charge transfer stacking with a ring-ring distance of 3.7 Å.

Atomic absorption analyses of the metal content of PLC (refs 12, 19 and 25) have consistently indicated two metal ions per mol of PLC and the same is true of a solution state EXAFS study<sup>26</sup>. The metal stoichiometry, however, was calculated from atomic absorption analysis using a relative molecular mass ( $M_r$ ) of 23,000 obtained from calibrated gel filtration<sup>27</sup>. Recalculation using the true  $M_r$  of 28,520 or 28,585, in the case of three Zn ions<sup>3</sup>, gives 2.3 Zn ions per mol PLC, indicating that the third metal site is at least partially occupied even in solution. Furthermore, both the analyses and the EXAFS study were carried out on PLC after an ion-exchange<sup>12,13,19,26</sup> or multi-stage dialysis treatment<sup>25</sup> to remove unbound metal ions, a treatment which would also remove loosely bound metal ions. By contrast, crystallization was performed in the presence of  $\sim 10 \mu\text{M Zn}^{2+}$ . □

**Note added in proof:** PLC has extensive homology with the first two-thirds of the recently published sequence of the alpha-toxin (phospholipase C) of *Clostridium perfringens*<sup>38-40</sup>.

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## ERRATA

### Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K<sup>+</sup> channel

Yoshihisa Kurachi, Hiroyuki Ito, Tsuneaki Sugimoto, Takao Shimizu, Ichiro Miki & Michio Ui

*Nature* **337**, 555-557 (1989).

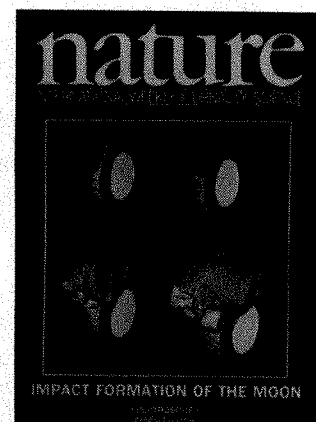
FIGURES 2 and 3 of this letter were inadvertently transposed during the editing process.

### Geochemical implications of the formation of the Moon by a single giant impact

H. E. Newsom & S. R. Taylor

*Nature* **338**, 29-34 (1989).

THE cover for the 2 March issue of *Nature* (shown right), illustrating the Review Article by Newsom and Taylor, was not properly credited. The computation to provide the image was done at Sandia National Laboratories, Albuquerque by Marlan Kipp and H. J. Melosh. Calculations were performed using the new 3-D code CTH on a Cray I XMP with a solid-state hard disk. The full computation required some 60 hours of computational time.



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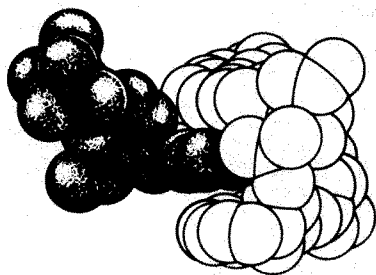
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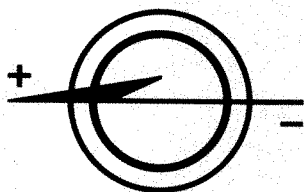
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## MRC LABORATORY OF MOLECULAR BIOLOGY NMR studies of HIV-1 tat

A 3 year post-doctoral post is available for research into the solution structure of the HIV transactivator protein. This forms part of a wider programme of studies of the structures and functions of HIV proteins in the Laboratory.

Experience in protein structure analysis by 2-D NMR would be preferred, but applicants with experience in other branches of NMR or other forms of structure determination would be considered.

Depending on experience, salary will be on the MRC grade II scales for non-clinical scientific staff (£11,070 to £14,500 per annum). Funds are available for immediate starting dates. Applications including a full curriculum vitae, a list of publications, reprints of no more than three relevant papers, and the names and addresses of 2 professional referees should be sent by 17 April 1989 quoting reference D/NMR to

**The Personnel Officer  
MRC Centre  
Hills Road  
CAMBRIDGE CB2 2QH**

**MRC**  
Medical Research Council

Further information is available from  
Dr Diana Dunstan or Dr David  
Neuhaus on (0223) 248011.

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(8829)A

## ПЕРЕВОДЧИКИ!

### FOR RUSSIAN-ENGLISH SCIENTIFIC AND TECHNICAL TRANSLATIONS

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(NW3233)A

## FACULTY POSITION IN PHYSIOLOGY

The Department of Physiology at Temple University School of Medicine invites applications for a tenure-track appointment at the ASSISTANT PROFESSOR level. Qualifications for the position include an M.D. or a Ph.D. degree with postdoctoral experience.

The applicant will be expected to develop an active independent research program supported by extramural funding and to contribute to the teaching programs of the department. All areas of research interest will be considered. Salary is commensurate with experience. Applicants should send curriculum vitae, description of research activities and the names and addresses of three references to:

**Dr. James P. Ryan, Chairman of  
Search Committee, Dept. of Physiology,  
TEMPLE UNIVERSITY SCHOOL OF  
MEDICINE, 3401 N. Broad Street,  
Philadelphia, PA 19140.**



Temple is an affirmative action, equal opportunity employer.

(NW3493)A

## IMAGE ANALYSIS PROJECT RESEARCH FELLOWS (2 positions) A\$31,005 p.a. – A\$45,177 p.a. CSIRO Division of Soils, Canberra, ACT AUSTRALIA

The Division seeks two scientists to work on the development of image analysis methodology and its application to a range of soil investigations. The appointee to Position One (No.A5289) will be expected to work in close collaboration with the CSIRO Division of Information Technology in the development of an image processing system for soil structural analysis. The second appointee (Position No. A3259) will have the background necessary to contribute to the estimation of soil structural properties using stereological and other mathematical techniques. Both candidates will be expected to use techniques developed to investigate soil structural problems associated with crusting, hard-setting and compaction of agricultural soils and other soil related materials.

Both positions are for a five year term; Australian Government superannuation benefits are available.

The appointees will have a PhD or equivalent in an appropriate scientific discipline and experience in image analysis methodology and its application.

Further details can be obtained from Dr. C.J. Chartres (6162) 46 5271 and duty statements and selection criteria from Ms. G. Morgan (6162) 46 5322; Fax (6162) 47 5883.

Applications including a full c.v. and the names of at least two referees should be directed to:

**The Chief  
CSIRO Division of Soils  
GPO Box 639  
Canberra ACT 2601  
Australia**

by April 17th 1989

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W5992)A



### SCIENCE EXHIBIT DEVELOPER

A position is available for someone to research topics in contemporary science, technology, and medicine and develop them into exhibits understandable and relevant to the general public. As part of a staff team, which conceives the theme and organization of the topics, the exhibit developer will contribute to the design presentation, originate and evaluate exhibit content, prototype, and test exhibit ideas. The developer will also utilize technologies, such as computers, to enhance the presentation of science information.

Requirements: A.B. or higher degree in a science or related field. Would be helpful to have excellent writing skills, experience in public education and project management, and skills to use computers and other kinds of exhibit equipment. Candidates should be imaginative, self-motivated, with hands-on laboratory and/or workshop experience. Send letter of application, CV, and three letters of recommendation to:

**Director of Human Resources,  
Museum of Science and Industry,  
57th Street and Lake Shore Drive,  
Chicago,  
IL 60637-2093. AA/EOE. (NW3495)A**

### UNITED MEDICAL AND DENTAL SCHOOLS of Guy's & St Thomas's Hospitals RESEARCH ASSISTANT

required in the Renal Unit of the Clinical Science Laboratories at our Guy's Campus (close London Bridge BR and London Transport) to work on a project involving molecular HLA mapping in patients with auto-immune disease. This is a collaborative project with a basic science laboratory in Oxford, to which occasional travel may be required. Duties include general laboratory responsibilities. Previous experience with DNA/RNA molecular biology techniques is desirable. Salary scale: £11,515 - £13,330 inclusive according to age, qualifications and experience. Appointment is for one year in the first instance. For further information, please telephone Dr. S. Sacks on 01-407 7600 extn 2784. To apply, please write with full CV and names and addresses of two referees to: **The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH, quoting Reference No. G/CSL/324. Closing date: 7 April 1989.** (8799)A

### UNIVERSITY OF SOUTHAMPTON Lymphoma Research Unit, Tenovus Laboratory Southampton General Hospital, Southampton, SO9 4XY

A post is currently available in the Tenovus Research laboratory for a

#### Research Assistant

to help establish culture facilities for the bulk production of monoclonal antibodies using hollow-fibre technology. The main objective of this project is to provide clinical grade material to a team of scientists and clinicians working on the production of novel antibody derivatives for the treatment of human lymphoma. Experience in cell culture technique and immunoassays would be desirable but not essential. The position is funded for three years and carries a salary, according to age and experience, on the University 1B scale for research staff (£8,675 - £11,680 pa, under review). For further details concerning the project contact Dr M Glennie at the Tenovus Research Laboratory (0703) 777222 Ext. 3230.

Applications including a C.V. and the names and addresses of two referees, to **Ms J A Doyle, Staffing Department, University of Southampton, Highfield, Southampton, SO9 5NH.**

Please quote reference 283/JAD/jm.  
(8794)A

### CHESTER BEATTY LABORATORIES INSTITUTE OF CANCER RESEARCH

#### Section of Cell and Molecular Biology A Postdoctoral Position

is available to work with Dr. Alan Hall at the Chester Beatty Laboratories to examine the function of the ras GTPase activating protein, GAP. A wide range of techniques will be used to study the biochemical and biological consequences of the interaction between ras and GAP. The work is part of a new collaborative venture with The Wellcome Foundation and the successful candidate will be able to make use of the excellent research facilities at the Chester Beatty Laboratories and at The Wellcome Research Laboratories at Beckenham.

Salary in the range £13130 to £15950.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

To apply, please submit a Curriculum Vitae, in duplicate, with the names and addresses of two referees to **The Personnel Officer, the Institute of Cancer Research, 17a Onslow Gardens, London SW7 3AL** quoting reference number 3.89.S.N.85. (8815)A

## RESEARCH FELLOW GEOPHYSICAL FLUID DYNAMICS

**A\$31,003 - A\$45,177**

**DIVISION OF ATMOSPHERIC RESEARCH,  
ASPENDALE, VICTORIA, AUSTRALIA.**

**FIELD:** Strait flows and Coastal Current Dynamics.

**THE DIVISION:** The Division of Atmospheric Research conducts strategic and applied research into problems concerning the physics, dynamics and chemistry of the atmosphere and ocean system.

**THE JOB:** The appointee will undertake research into the character of the stratified Bass Strait overflow and its consequences for the Tasman Sea circulation and non-linear coastal hydraulics generally, using laboratory and theoretical techniques. Studies of coastal systems and boundary currents affected by both stratified or Rossby-wave hydraulics are planned as an extension of this work.

**THE PERSON:** Applicants should possess a Ph.D degree or equivalent qualification in geophysical fluid dynamics, together with experience in or an aptitude for laboratory studies.

**CONDITIONS:** Appointment will be for a term of 3 years, with Australian Government superannuation benefits available.

**MORE INFORMATION:** Prospective applicants are invited to contact Dr. Peter Baines on (0011-61-3) 586 7651, fax (0011-61-3) 586 7600, for further information. Dr. Baines can also provide a copy of the duty statement and selection criteria.

**APPLICATIONS:** Applications should be submitted by 14 April 1989 and should quote reference number A1848. They should be framed against the selection criteria and should provide relevant personal particulars, including details of qualifications and experience. Applicants should nominate at least two professional referees and address their application to:

**The Chief,  
CSIRO Division of  
Atmospheric Research,  
Private Mail Bag No. 1,  
Mordialloc, Victoria, Australia 3195.**



**CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W5993)A**  
AT 89/09a

### UNIVERSITY OF TASMANIA

Applications are invited from suitably qualified men or women for the following position.

#### CHAIR OF PHYSIOLOGY (Ref. 42/89)

Applications are invited for appointment to the Chair of Physiology and Head of Physiology, a position which has been vacant since the retirement of Professor A. F. Cobbold.

The Department of Physiology includes Pharmacology and is an integral part of the Faculty of Medicine. It is also a member of the newly-formed School of Biological Sciences.

The Department has responsibility for the undergraduate teaching of physiology to medical and pharmacy students and pharmacology to medical students. Postgraduate degree courses in Master of Medical Sciences and Doctor of Philosophy are offered within both the physiology and pharmacology disciplines.

Expressions of interest are welcome from applicants with a high research standing, wide experience in postgraduate supervision and an experimental orientation towards any area of physiology and/or pharmacology. The successful applicant will be expected to provide teaching, research and administrative leadership within the Department.

An information booklet containing details about the post, the Faculty of Medicine and the University is available from the Staff Office.

Enquiries about academic aspects of the post should be directed to the Head of the Department of Physiology, Dr. L. J. McLeod (tel. 002.202678).

The Professorial salary effective from 15 May is \$63,919 with a clinical loading of between \$5,690-\$11,366 for a medically qualified appointee. The appropriate loading depends on the extent of clinical responsibilities undertaken.

Applications close 31 May 1989.

Position information and application forms available from the Staff Office Secretary, tel. (002) 202013. Applications quoting ref. should be sent to the **Staff Officer, University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania, Australia, 7001.**

**THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER (W6000)A**



# UNIVERSITY OF ABERDEEN

## New Academic Appointments Scheme

Applications are invited for the following new posts under the terms of the UGC Scheme. The intention is to recruit younger academic staff and it is anticipated that initial appointment will be to the Lecturer Grade A Scale (£9260 - £14500). The University expects to be able to offer lectureships in the following areas from October 1989/90:

### Forestry/Plant Science

**Ref. JA/035** Research is expected to concentrate on host identification and infection in fungal diseases of trees. Applicants should have a background in either Forestry or Plant Science with an interest in the other area.

### Biochemistry

**Ref. JA/036** Research will concentrate on the area of macromolecular biochemistry with special reference to the nucleic acid: protein interface in eukaryotic systems.

### Bio-Medical Physics/Computing Science

**Ref. LW/006** Complementing current research activities in both Departments, the research specialisation will be "The Application of Parallel Systems to Vision/Medical Imaging".

### Biotechnology

**Ref. JA/039** Applicants for these 2 posts should have primary training in recombinant DNA technology with research interests in one of the following areas: microbial plant pathogenesis (bacterial/fungal); molecular parasitology or insect molecular biology; control of gene expression in lower eukaryotes; or molecular immunology.

### Chemistry

**Ref. JA/037** Applicants should have research interests in any branch of physical chemistry. Special consideration will be given to those with expertise in the area of spectroscopy/molecular structure, kinetics or polymer science.

### History/History of Art

**Ref. JA/038** The appointee will specialise in the late medieval/early modern European period.

### Spanish

**Ref. JA/041** Applicants should have research interests in Latin-American and modern Spanish literature. An interest in language-teaching methodology will be a strong additional qualification.

### Physiology

**Ref. LW/008** Applicants should have a research interest in neuroscience, particularly in autonomic control, and experience in modern electrophysiological and molecular probe techniques.

### Zoology

**Ref. LW/009** Applicants should have extensive research experience in the use of standard respirometry and stable isotope techniques, particularly doubly-labelled water, with a view to their application in the study of marine resources and the energetics of marine top predators.

### Geography

**Ref. JA/040** Research in the field of Remote Sensing, with a subsidiary specialism in Geographical Information Systems. Involvement in a group research programme in the area of mapping science. Teaching will relate mainly to the established MSc course in Environmental Remote Sensing.

### Public Law

**Ref. LW/007** Applicants should have a particular interest in European Law.

### Divinity

**Ref. LW/010** Applicants should have a research interest in one or more of the following areas: Third World issues, business and medical ethics, mission and ecumenism.

Further particulars may be obtained from the Personnel Office, University of Aberdeen, Regent Walk, Aberdeen, AB9 1FX (Telephone 0224 273500) to whom applications (2 copies) should be returned by 21 April 1989. Please quote the appropriate reference number.

(8788)A

## University of Glasgow Department of Physics and Astronomy Experimental Particle Physics

### KELVIN CHAIR

Applications are invited from experimental particle physicists for appointment to the Kelvin Chair in the Department of Physics and Astronomy. The appointment will be tenable from 1st October 1989 or as soon as possible thereafter. The successful candidate will have an outstanding research record, have demonstrated leadership abilities and will be expected to carry out research in experimental particle physics and to contribute to undergraduate and postgraduate teaching in physics.

Further particulars may be obtained from the Academic Personnel Office, University of Glasgow, Glasgow, G12 8QQ (Tel. 041 339 8855) where applications (3 copies; 1 copy in the case of overseas applicants) giving the names and addresses of three referees should be lodged on or before 2nd May, 1989.

In reply please quote Ref. No. 6506M. (8792)A

## UNIVERSITY OF AUCKLAND New Zealand

DEPARTMENT OF CELLULAR AND  
MOLECULAR BIOLOGY

### POSTDOCTORAL POSITION

To study the effects of cyclic AMP on growth and differentiation of tumour cells, particularly effects on phosphatidylinositol metabolism and possible involvement of oncogenes. Experience in these areas would be advantageous. The position is available for 2 years and includes a return airfare. For further information contact Professor Ray Ralph, Department of Cellular and Molecular Biology, University of Auckland, Private Bag, Auckland, New Zealand. Tel: 64 9 737 999, Fax: 64 9 31 618. (W5995)A

## ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London) Postdoctoral Pharmacologist

Applications are invited for a postdoctoral research worker for a three year appointment in the Department of Physiology. The project involves investigating the receptor mechanisms which control airway secretion. Salary will be on the RIA scale (£11,515 - £17,370, including London allowance) depending on qualifications and experience.

### Research Technician

Applications are invited for a post as research technician, grade 5, for a three year appointment to assist in the above project. Salary up to £9,088 (including London allowance).

Application forms and further details from the Personnel Office, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE (01-672 9944 ext 56020).

Closing date 14 April 1989. Quote reference 40/89.

(8797)A

## UNIVERSITY OF CAMBRIDGE University Lecturer in Immunology in the Department of Pathology

University Lecturer in Immunology to take up appointment on 1st October 1989, or as soon as possible thereafter. The successful candidate will be expected to have an interest in basic mechanisms in Cellular and Molecular Immunology and to offer a research programme complementary to those ongoing in the Division and to contribute to the Departmental teaching.

Salary ranges: £13,365 to £20,615.

Further information and application form from:

**The Secretary, Appointments Committee,  
19 Trumpington Street, Cambridge, CB2 1QA**  
(Tel: 0223 334999 Fax: 0223 332355).

Closing Date: 12 May 1989 (8817)A

## The Center for Climate Research of Lamont-Doherty Geological Observatory

The Center for Climate Research of Lamont-Doherty Geological Observatory has received a gift to be used for construction and exercise of a global ocean simulation model for climate change research. The model will test scenarios of natural variability and human impacts, including those evident in the paleoclimate record.

*We will create an outstanding scientific group by adding the following personnel.\**

■ **Ocean Modeler.** Either a numerical analyst using these skills to solve problems in ocean physics, or an oceanographer well versed in numerical techniques. An ocean model suitable for climate studies must be able to withstand a confrontation with observational data: physical, chemical, biological, and paleo — though not necessarily on the first try. The arrogance to blame discrepancies on the forcing or verification data can be a plus, but only if it can be rigorously justified and is accompanied by a suggested solution. As you see present ocean models, what are their principal problems? Your solutions?

■ **Empirical Studies** of the global ocean relating to climate. An oceanographer or meteorologist whose primary interest is data, but who is enthusiastic about using data in conjunction with numerical models. Sufficiently interested in nuts and bolts issues to direct an effort to create, manage, and provide the tools to manipulate the oceanographic and atmospheric database needed to initialize, drive, and verify ocean models. What questions do you think can be answered with the data on hand?

■ **El Niño Forecaster.** A meteorologist or oceanographer to assume the leadership of our ongoing ocean-atmosphere dynamical prediction activity. The opportunity to choose the direction of future research, including an expansion to climate phenomena other than ENSO. Skills in statistical methods and empirical studies would best complement our strengths in theory and modeling.

■ **Scientific Programmer.** Builder/Keeper of the global ocean model. Experience with large scale numerical models and supercomputers essential.



Applications should be sent to: Office of the Director  
**Lamont-Doherty Geological Observatory of Columbia University**  
Route 9W / Palisades, NY 10964  
*Columbia University is an Equal Opportunity/Affirmative Action Employer*

### POST DOCTORAL FELLOWSHIPS:

#### ■ Ocean-Atmosphere Interaction.

To study and model the Planetary Boundary Layer or the Ocean Mixed Layer. We are especially interested in the tropics and high latitudes.

#### ■ Physical or Chemical Oceanography.

To diagnose model problems by intensive study of a specific physical oceanographic problem crucial to an understanding of the global ocean circulation. What problem would you like to work on?

#### ■ Ocean-Atmosphere Interaction.

To build on present dynamical modeling studies of ENSO. Possibilities include extension of the model to the global tropics; study of tropical flood/drought, e.g. in Brazil, Africa, India; analysis of related observational data.

*\*(Ph.D. or equivalent experience required. Level of appointment will be commensurate with education and experience.)*

(NW3489)A

## SEARCH RE-OPENED TENURE-TRACK FACULTY POSITION IN PALEOCEANOGRAPHY

The Institute of Marine Science, University of Alaska Fairbanks, has re-opened its search for a tenure-track faculty member in paleoceanography. The appointment will be with the Institute of Marine Science in the recently organized School of Fisheries and Ocean Sciences and involves research, graduate-level instruction, and direction of graduate student research. The Institute of Marine Science has a faculty of 24 and about 40 graduate students at the M.S. and Ph.D. level. Institute faculty are involved in a variety of research programs emphasizing arctic and sub-arctic seas and are actively planning university-wide programs addressing global change.

We seek a colleague committed to collaborative work in paleoceanography in a broad sense and welcome applicants bringing modern approaches from geology, physics, chemistry or biology. The appointee must hold a Ph.D. and will be responsible for developing an active research program in paleoceanography. We anticipate filling this position at the assistant professor level, but will consider a more senior appointment in exceptional cases.

Applicants should send (by April 30, 1989) a statement of research interests, a curriculum vitae, and the names and addresses of three references to:

**William S. Reeburgh**  
**Chair, Paleoceanographer Search Committee**  
**C/O Director's Office**  
**Institute of Marine Science**  
**University of Alaska Fairbanks**  
**Fairbanks, Alaska 99775-1080**

Applications of finalists may be subject to public disclosure. Persons hired must comply with the new employment provisions of the Federal Immigration Reporting Act. The University of Alaska is an Equal Opportunity/Affirmative Action Employer, educational institution, and could become a year-round paradise with a little global warming.

(NW3470)A

## UNIVERSITY OF NEWCASTLE UPON TYNE THE MEDICAL SCHOOL

### Lecturer in Environmental Toxicology

Applications are invited for a new post of Lecturer in Environmental Toxicology in the Division of Environmental and Occupational Medicine, which we expect to be able to fill under a new UGC initiative. A suitable candidate would have a research background in chemistry or other life science and a higher degree and be interested in the application and development of analytical techniques for monitoring toxic compounds in the environment and man. A teaching commitment would be expected on an established MSc course in Occupational Hygiene and a new course in Human Toxicology. Participation in the provision of a specialist analytical service to industry through the Medical School Company would be encouraged.

Closing date: 14th April 1989.

### DEPARTMENT OF PHYSICS

#### Lecturer in Computational Solid State Physics

Applications are invited for the above post which we expect to be able to fill from October 1989 under a new UGC initiative. Current research efforts in the field concentrate on electronic structure, optical and structural properties of perfect and imperfect solids, with particular emphasis on low dimensional semiconductor microstructures. The computing facilities available on the campus include Amdahl 5860, Encore Multimax and Gould NPI with vector processing facility. There are collaborative contacts with prestigious research laboratories engaged in related experimental work. This research programme enjoys generous support from funding agencies in the UK, USA and European Community. Applicants should be able to demonstrate significant research and achievement in a closely related or complementary research activity.

Closing date: 21st April 1989.

#### FOR BOTH POSTS:

Salary will be at an appropriate point on either Lecturer Grade A: £9,260-£14,500 or Grade B: £15,105-£19,310 p.a. according to qualifications and experience. This appointment is viewed as an opportunity for candidates in the early stages of their careers.

Further particulars may be obtained from the **Senior Assistant Registrar (Establishments), The University, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU** with whom applications (3 copies) with the names and addresses of three referees.

(8827)A



# RESEARCH FELLOW (APPLIED CLIMATOLOGIST)

\$A31,003-\$A45,177

DIVISION OF ATMOSPHERIC RESEARCH  
ASPENDALE, VICTORIA, AUSTRALIA

**THE PROGRAM:** The Division of Atmospheric Research has a major program investigating regional climate change and its impacts, with special reference to the greenhouse effect.

**THE JOB:** To undertake research into the potential impact of the greenhouse effect at a local and regional level, using the best available scientific information from climate modelling and other sources, with a view to providing state-of-the-science advice to local and regional authorities and industries. Liaison with other scientists, specialists and potential users of information across a range of disciplines will be required.

**THE PERSON:** Applicants should possess a Ph.D. degree or equivalent in meteorology or the atmospheric sciences, and preferably, a strong background of experience in applied climatology, computing, and interaction with industries and public authorities. Consideration could be given to the appointment of an outstanding applicant at a higher level.

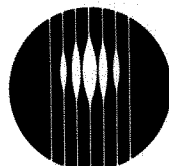
Maturity of judgement, an ability to write succinct and well-documented reports, and to communicate across disciplines and to the media is essential. The appointee will be located at Aspendale, but must be prepared to travel interstate.

**CONDITIONS:** Appointment will be for a term of 4 years, with the possibility of a further term. Australian Government superannuation benefits are available.

**MORE INFORMATION:** Prospective applicants are invited to contact Dr A. Barrie Pittock on (0011-61-3) 586 7527, fax (0011-61-3) 586 7600, or telex AA34463, who can provide a copy of the duty statement and selection criteria.

**APPLICATIONS:** Applications should be submitted by 14 April 1989 and should quote reference number A1839. They should be framed against the selection criteria and should provide relevant personal particulars, including qualifications, transcripts of tertiary results and experience. Applicants should nominate at least two professional referees and address their application to:

The Chief,  
CSIRO Division of  
Atmospheric Research,  
Private Mail Bag No. 1,  
Mordialloc, Victoria, Australia 3195.



CSIRO  
AUSTRALIA

(W5991)A

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

AT 89/088

## THE LONDON HOSPITAL MEDICAL COLLEGE (University of London) RESEARCH ASSISTANT (1B) The Medical Unit

Research Assistant required to join established molecular biology group working on the immunogenetics of kidney disease. Would preferably suite a graduate in biochemistry, genetics, immunology or microbiology. Previous experience of molecular biology techniques would be an advantage. Salary £8,675 plus £1,650 London Weighting.

Application form and job description available from Dr G A Hitman, Assistant Director, Medical Unit, The London Hospital, Whitechapel, London E1 1BB (Tel: 01-377 7111).

Closing date: within 2 weeks of the date of this advertisement. (8802)A

## M.D. ANDERSON CANCER CENTER

Postdoctoral and Research Associate Positions, M.D. Anderson Cancer Center, Department of Clinical Immunology and Biological Therapy in the Laboratory of Cytokine Research. Identification of new cytokines (growth inhibitors and stimulators) produced by the immune system and involved in tumor cell surveillance. Interested in understanding the role of oncogenes, signal-transduction and growth factors in the mechanism of tumor cell resistance to these cytokines. Experience in cell biology, protein purification or gene cloning; background in cellular immunology and/or tumor biology is desirable. Ph.D. and/or M.S./B.S.

Please send C.V. with the names of three references to: Dr. Bharat B. Aggarwal, Chief, Section of Cytokine Research, M.D. Anderson Cancer Center, Department of Clinical Immunology and Biological Therapy, 1515 Holcombe Boulevard, Houston, Texas 77030.

EOE/AA

(NW3479)A

## NATIONAL INSTITUTE FOR MEDICAL RESEARCH GENES AND CELLULAR CONTROLS GROUP RESEARCH OFFICER/SENIOR RESEARCH OFFICER

Applications are invited for a Research Officer/Senior position tenable in the laboratory of Eukaryotic Molecular Genetics (Head: Dr P W J Rigby). The person appointed will work with Dr Rigby on the construction of retrovirus vectors and their use in analysing gene expression in cultured embryonic stem cells in embryos, and on other projects concerned with the regulation of gene expression in tumour and embryonic cells. Previous experience in recombinant DNA technology is essential; experience with mammalian cell culture, virology or embryology would be advantageous.

Qualifications could include a degree, HTEC or similar, but enthusiasm, ability to accept responsibility and work independently are more important.

Entry to the Research Officer grade is subject to two years relevant experience after graduation. Applicants with less than two years experience will initially be appointed to the Trainee Research Officer grade. Salary is in the range of £8,172 (for immediate graduates) to £14,646 per annum inclusive of London Weighting.

The Institute is situated in pleasant rural surroundings and offers good sports, recreational and social facilities. Please telephone for an application form on 01-959-3666, extension 2270, or write to Mr N J Bowry, Personnel Officer, NIMR, The Ridgeway, Mill Hill, NW7 1AA quoting reference: GEKG/0368.

The closing date for completed applications will be 21st April 1989

An Equal Opportunities Employer. (8814)A

MRC

## INSTITUTE OF ZOOLOGY ZOOLOGICAL SOCIETY OF LONDON

**RESEARCH ASSOCIATESHIP** (Ref: ADB/NLCM). A postdoctoral developmental biologist/molecular geneticist is required in the Developmental Biology Unit to join a team investigating endocrine functions of the primate blastocyst, in particular the interaction between inner cell mass tissue and trophoblast in the secretion of chorionic gonadotrophin. The post involves close collaboration with the Molecular Genetics Unit and with others studying the endocrinology of early gestation in the marmoset. This position would suit a developmental biologist wishing to apply molecular genetic techniques to problems of early embryonic development, or a molecular geneticist wishing to investigate this area. The post which is funded by an MRC/AFRC Programme Grant, is for 3 years in the first instance, with the possibility of extension for a further 2 years. Salary in the range of £11,448 - £13,263 including London Weighting, according to age and experience. For further details please contact Professor A P F Flint, Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY (telephone: 01-722 3333), to whom written applications, including a detailed CV and the names of three referees, should be sent not later than 21 April, 1989.

(8786)A

## JUNIOR INVESTIGATORS RESEARCH ASSOCIATESHIPS POSTDOCTORAL FELLOWSHIPS

### UNIVERSITY OF OTTAWA HEART INSTITUTE

The newly established University of Ottawa Heart Institute Research Centre has openings for the above positions in the field of receptors, signal transduction, regulation of gene expression as well as the biochemistry of cardiac and connective muscle tissue.

The centre offers excellent new physical facilities as well as the possibility of direct interaction with the clinical staff.

*In accordance with Canadian Immigration requirements, priority will be given to junior investigators and research associates who are Canadian citizens or landed immigrants in Canada.*

Submit curriculum vitae, statement of research interests, and the names, addresses, and telephone numbers of three references to: Dr. Adolfo J. de Bold, Director of Research, University of Ottawa Heart Institute, 1053 Carling Avenue, Ottawa, Canada, K1Y 4E9. (NW3492)A

**HARVARD MEDICAL SCHOOL  
MASSACHUSETTS GENERAL —  
McLEAN HOSPITALS**

**CHIEF OF PSYCHIATRY**

Harvard Medical School and the Department of Psychiatry at the McLean and Massachusetts General Hospitals are seeking an internationally renowned psychiatrist who possesses the clinical experience, administrative skills and management competence necessary to oversee a complex network of inpatient and outpatient services. The candidate should be a noted investigator and teacher with broad experience and skills as an academic psychiatrist who meets the criteria for a tenured professorship at HMS. He/She should be committed to a major research program aimed at understanding the cause, prevention and treatment of mental illness. The individual selected will hold titles of Professor of Psychiatry and Head of the Massachusetts General Hospital-McLean Hospital Department of Psychiatry at the Harvard Medical School; Chief of the Psychiatric Services at the Massachusetts General Hospital; Psychiatrist-in-Chief and General Director of the McLean Hospital. Reply with curriculum vitae to **Dr. Arthur Kleinman, William James Hall 330, 33 Kirkland Street, Harvard University, Cambridge, MA 02138.**

*Harvard Medical School, Massachusetts General Hospital, and McLean Hospital are Affirmative Action/Equal Opportunity Employers.* (NW3490)A

**STATE OF KUWAIT  
KUWAIT UNIVERSITY  
COLLEGE OF SCIENCE**

The Kuwait University College of Science, Dept. of Botany & Microbiology invites applications for posts of Professor, Associate Professor and Assistant Professor for the academic year 1989/90, tenable February 10, 1990 in the following disciplines:—

- **Plant Breeding**
- **Microbial Physiology**
- **Agriculture & Economic Botany.**

The language of instruction of the College of Science is English. The method of teaching is based on the credit hour system. Applicants must hold the Ph.D. or its equivalent at the time of application.

All applications together with a non-returnable copy of academic Qualifications and representative publications should be sent by registered post directly to:—

Dean  
College of Science  
P.O. Box 5969  
13060 Safat  
Kuwait

*All applications must be received by August 31, 1989.* (W5997)A

**GBF Gesellschaft für Biotechnologische Forschung mbH,** Braunschweig, FR Germany — a government supported Research Institute of Biotechnology — offers a

**Postdoctoral Position**

The appointed scientist will join an international collaborative project involving Dr. J.E.G. McCarthy (GBF), Dr. M.F. Tuite (Kent Univ., England) and Dr. A.J.P. Brown (Glasgow Univ., Scotland), financed by the Commission of the European Communities.

The position is available for three years from May 1989. Applications from candidates with experience in (yeast) molecular genetics should be addressed (referring to ref. no. 40/89) to the Personnel Dept., GBF, Mascheroder Weg 1, D-3300 Braunschweig, FRG. Please include a complete c.v., list of publications, details of research activities to date and the names and addresses of three referees. Informal enquiries can be made to Dr. J.E.G. McCarthy (Tel. 0531/6181-430).

(W5999)A

# Pharmaceutical Research

We are one of the largest French pharmaceutical firms recognized internationally for the efficiency of our achievements in research. We are currently establishing new research initiatives and are recruiting scientists with experience in drug discovery for the positions of Research Department Director and Project Leader in the following therapeutic specialities.

## Hæmobiology

**Haematologists knowledgeable in the mechanisms of coagulation and the interaction of blood elements with the vascular wall relevant to the aetiology of atherosclerosis.**

## Phlebobiology

**Candidates highly competent in haemodynamics, vascular inflammation and haemostasis.**

Candidates must have a Ph.D. or equivalent degree and have extensive experience of pharmacological models relevant to each speciality.

The successful candidates will be inventors, combining the qualities of scientist and industrialist, and will be capable of applying their ideas to the discovery of new compounds.

Experience in the pharmaceutical industry is desirable and the ability to interact constructively with therapeutic chemists is essential.

Our research centre is located in suburban Paris.

Please forward a hand-written letter, curriculum vitae and photograph to Media-System, 6 impasse des Deux Cousins, 75849 Paris cedex 17 - France.

Please quote ref. 49464.

(W5998)A

## A LINK PROGRAMME

### PROGRAMME MANAGER

#### COLLABORATIVE RESEARCH IN BIOCHEMICAL ENGINEERING

A major LINK Programme of Collaborative research in Biochemical Engineering is to be launched in the spring of 1989. It will be funded by UK industry, DTI and SERC, and will comprise many inter-related projects, involving UK firms and UK research organisations, in collaborative research.

It is necessary to appoint a Programme Manager, who will have a major role in establishing and managing the Programme. This role will involve, inter alia, stimulating collaborative proposals and coordinating the activities of the research groups involved. He/she would also be responsible for ensuring effective interaction between all the funding partners. Candidates should preferably have experience of equipment and process development in the biotechnology field.

This full-time, fixed term appointment will be for 3 years initially, with possible extension, and location will be by arrangement, although considerable travel will be required. Salary is negotiable. Secondment from industry or academia would be considered.

Please send CV and statement describing applicant's suitability for the post by 20th April 1989 to:

Mr R A Frewin, (8826)A  
Branch 3,  
Engineering Markets Division,  
Room 365, Ashdown House,  
123 Victoria Street,  
London SW1E 6RB

serc



dti

The Science and Engineering Research Council

#### University of Geneva announces an opening for a position of a FULL PROFESSOR in

#### COMPUTATIONAL CONDENSED MATTER PHYSICS

The new professor will have the responsibility for research and teaching in computational condensed matter physics. He will in particular have the task to develop the research activities of the "Institut Romand de Recherches Numériques en physique des Matériaux" (IRRMA) situated at the campus of the "Ecole Polytechnique Fédérale de Lausanne". He will assume the direction of this Institute for a certain number of years and it is also expected that he actively participates in the scientific and academic life of the University of Geneva.

The applicant should have a Ph D in physics or an equivalent degree. Experience in teaching, leading and management of research groups as well as some experience in university administration is desirable. Letters of application, a curriculum vitae and a list of publications should be addressed before June 30 1989 to:

Secretariat de la Faculté des Sciences,  
20, quai Ernest-Ansermet,  
CH-1211 Geneva 4, Switzerland

where additional information may be obtained. (W5986)A

#### ASSISTANT PROFESSOR

#### QUANTITATIVE EPIDEMIOLOGY OF PLANT PATHOLOGY

Applications are invited for a tenure-track position to conduct independent and collaborative research on the quantitative epidemiology of plant pathogens. Teaching duties include development of a graduate course in quantitative epidemiology. A Ph.D. with demonstrated expertise in quantitative biological research is required. Postdoctoral experience is preferred. Send curriculum vitae, transcripts, selected reprints, a statement of research interests and three letters of reference to: Dr. A. H. Epstein, Iowa State University, Dept. of Plant Pathology, Ames, IA 50011-1020, 515-294-1741. Deadline: May 15, 1989, or until position is filled.

Affirmative Action/Equal Opportunity Employer. (NW3497)A



#### Position announcement

GEOMAR, Research Center for Marine Geosciences which was established in 1987 at the Christian-Albrechts-Universität in Kiel/Germany, invites applications for a C-1 appointment in

#### Marine Environmental Geology

C-1 is the classification in the German civil service system equivalent to Assistant Professor.

The successful candidate will be expected to develop a research program on natural recycling processes at the seafloor as well as participate in the build-up of the Research Center. Desired expertise in one or more of the specialties: trace elements, biomarkers, organic-mineral interaction.

Ph.D. or equivalent degree is required. Women are encouraged to apply. Please send applications, including resumé and addresses of three references, before May 15, 1989 to:

GEOMAR  
Research Center for Marine Geosciences  
Personnel Department  
Wischhofstrasse 1-3  
D-2300 Kiel 14  
Fed. Rep. Germany

(W5988)A

#### MOLECULAR VIROLOGY FULL PROFESSOR (C4) UNIVERSITY OF MÜNSTER FR – Germany

The University of Münster is establishing a clinical research center for 'Molecular Biology of Inflammation'. It will finally comprise five new Institutes and five associated research groups. The first position (tenure track, full professorship) is now offered to an experimental molecular virologist. The successful candidate will be the director of the newly founded institute with due regard to the regulations of § 29 Wiss. HG.

Applicants should have a background in either Herpes virus or retro-virus research (e.g. AIDS). Participation in regular basic teaching in virology is expected. Applicants from abroad are encouraged.

Please send curriculum vitae, bibliography, an outline of research interests and of teaching experiences to the:

Dekan der Medizinischen Fakultät  
Domagkstraße 3  
D – 4400 Münster  
FR – Germany

Closing date: 12.06.1989

Telefon: 01049251/83 5490

Telefax: 01049251/83 6960

(W5987)A



#### Forestry Research Coordination Committee

FRCC

#### FARM FORESTRY

At least £450k will be available over 3 years for research into the environmental, economic and social aspects of farm forestry. This Special Topic Programme is being launched by the Forestry Research Coordination Committee and will be jointly funded by the Department of the Environment, the Forestry Commission and the Natural Environment Research Council. Applications for research grants and research studentships are invited from centres of Higher Education and recognised research institutes.

Further details and application forms from:  
Colin Barr, NERC, Polaris House,  
North Star Avenue, Swindon SN2 1EU.  
Closing date for applications: 28 April 1989.



(8825)A



# THE HANNAH RESEARCH INSTITUTE

## IMMUNOLOGIST/ BIOCHEMIST

An immunologist or a biochemist with experience in immunology is required to investigate the autoimmune control of milk secretion. The appointee will join a team within the Director's Research Group which is studying local mechanisms regulating mammary function. Techniques will include EPLC, polyclonal and monoclonal antibody production, and radioimmunoassay and enzyme immunoassay development. The post is funded for 2 years.

Applicants should possess a first or upper second class degree in immunology, biochemistry or a related discipline, with at least 2 year's relevant experience.

Appointment will be at Higher Scientific Officer grade, with starting salary up to £10,994. A non-contributory superannuation scheme is operating.

Further details may be obtained from The Secretary, The Hannah Research Institute, AYR KA6 5HL to whom applications, including the names of two referees should be forwarded by 8 April 1989 quoting reference HRI/165. (8801)A

# UNIVERSITY OF DURHAM DEPARTMENT OF CHEMISTRY SENIOR RESEARCH ASSISTANT IN ORGANIC CHEMISTRY

Applications are invited from organic chemists with a Ph.D. degree for work on the synthesis of novel materials containing fluorocarbon units, with potential wide applications. Work will be supervised by Professor R. D. Chambers and carried out in close collaboration with industry. Background experience in free-radical reactions or polymer chemistry would be useful but not essential. The post is tenable from March 1989 for one year but renewal for up to three years is anticipated.

Salary will be in the range £9,865-£11,070 per annum.

Applications (three copies) naming three referees should be sent to the Registrar, Science Laboratories, South Road, Durham, DH1 3LE, as soon as possible. For further information telephone Professor R. D. Chambers (tel. (091) 374-3120). (8795)A

## Develop your research skills - and career - in a pioneering Research Unit

# Scientist/Senior Technician

An impressive range of well established pharmaceuticals has won Ciba-Geigy the respect of colleagues and competitors throughout the industry. Our reputation for innovation is exemplified by our Advanced Drug Delivery Research Unit, presently working on designing means of achieving site-specific delivery of conventional and genetically defined drugs. It is in this Unit that we need a Scientist/Senior Technician in the cell biology research area.

If you're keen to work on projects of considerable challenge and potential, you'll be interested in joining us at our superbly equipped laboratories in Horsham.

We operate a flexible career structure, allowing senior technicians to move up into scientific grades. So you may be a young Graduate, with up to 2 years' experience, or an HNC/HND qualified professional with a solid background in a similar industrial or public sector environment. Either way, you'll be self-motivated, and be equally capable of working on your own or within a multi-disciplinary team. A practical 'hands on' approach is crucial.

Familiarity with cell culture, biochemical and/or immunological analytical techniques is particularly relevant, because you will be helping to determine epithelial transport pathways for macromolecules in vitro, as well as the evaluation of novel delivery systems.

Along with outstanding prospects for personal and professional development, you'll receive a competitive salary backed by a full range of major company benefits.

Please write, enclosing your full C.V. quoting reference V41 to: The Personnel Department, Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex RH12 4AB. Tel: (0403) 50101.

# CIBA-GEIGY

(8831)A

## HERIOT-WATT UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES LECTURER

Applications are invited for a lectureship under the New Academic Appointments Scheme designed to recruit younger academic staff.

This is not a fixed term appointment and it will be associated with the International Centre for Brewing & Distilling and the lecturer will be expected to carry out teaching and research in areas of relevance to the malting, brewing and distilling industries. Preference will be given to candidates with experience of research at a post-doctoral level in either plant or yeast molecular biology.

The salary will be on the Lecturer A scale £9,260-£14,500 (under review). Further particulars and application forms are available from the Staff Officer, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS. Please quote ref no 26/89/N. Closing date 21st April. (8809)A

## CENTRAL VETERINARY INSTITUTE Lelystad, The Netherlands

### The Department of Virology seeks to recruit 2 MOLECULAR BIOLOGISTS

to work in a research group dealing with the construction of a vaccine based on a deletion mutant of bovine herpesvirus type 1, that also serves as an expression vector of genes for bovine respiratory virus.

The posts are funded for a period of 4 years.

Candidates with a Ph.D. degree in the biological sciences and a broad experience in DNA-recombinant technology are invited to apply.

Salary is within the range of Dfl. 50.000-80.000 (approx. \$23.000-36.000) per year.

For enquiries phone Dr. J.T. Van Oirschot (03200-73911, ext. 308), or Dr. A.L.J. Gielkens (03200-26814).

Application with full curriculum vitae and names of 2 referees should be submitted within 3 weeks to the Personnel Office, The Central Veterinary Institute, P.O. Box 65, 8200 AB Lelystad, The Netherlands. (W6001)A



Department of Scientific and  
Industrial Research



## SCIENTIST-EMBRYOLOGY

Biotechnology Division

(Ref no: Bio 14)

A vacancy exists in an expanding group using cell culture techniques to produce embryos for a transgenic ruminant animal programme.

A scientist is needed who has experience in vitro maturation and fertilisation of ruminant oocytes and embryo culture.

This person will be actively involved in collaborating with molecular biologists to produce transgenic animals.

Applicants should possess a Ph.D. in physiology, biochemistry, animal or veterinary science.

The applicant for this position should be able to work effectively in a multi-disciplinary group.

The position is for three years initially.

Salary will be commensurate with qualifications and experience.

Please apply in writing and enclose a Curriculum Vitae including the names of at least two referees by 14 April 1989 to:

The Personnel Officer  
DSIR, Private Bag  
Palmerston North  
New Zealand.

(W5994)A

### UNIVERSITY OF LONDON CHAIR OF GEOLOGY TENABLE AT BIRKBECK COLLEGE

The Senate invite applications for the above Chair tenable from 1 October 1989.

Applications will be considered in all branches of geology/geophysics. The successful applicant will have demonstrated high research capabilities and potential, and will be expected to set up and lead a research team in his/her own field. He/she will also contribute as appropriate to teaching on BSc and MSc courses and to departmental administration.

Applications (10 copies) should be submitted to the **Teachers' Section (N), University of London, Senate House, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained.**

The closing date for receipt of applications is 28 April 1989. (8800)A

### ATMOSPHERIC PHYSICIST

to carry out field and laboratory studies related to cloud radiation and electrification processes. Duties include aircraft and cloud data reduction and analysis; design and construction of measurement systems; and program development. Requires Ph.D. in Physics or related discipline, with experience in aircraft and/or laboratory cloud physics/chemistry; working knowledge of electronics and computing related to aircraft operations and/or laboratory instrumentation. Position will be filled at either the Assistant or Associate Professor level, rank and salary dependent upon qualifications.

Applications must be received by 5/31/89.

Send resume and letter of interest to Personnel Office, Desert Research Institute, University of Nevada System, P.O. Box 60220, Reno, NV 89506.

An Affirmative Action/Equal Opportunity Employer.

(NW3502)A



ROYAL HOLLOWAY AND BEDFORD

NEW COLLEGE

University of London

School of Life Sciences

DEPARTMENT OF BIOCHEMISTRY

### TWO LECTURESHIPS IN PLANT BIOCHEMISTRY MOLECULAR BIOLOGY

Applications are invited for these two posts in the Plant Exploitation and Protection Group, in association with the appointment of Dr Bowyer to the Chair in Plant Biochemistry. The main research interests of the Group are the structure and assembly of Photosystem the biosynthesis of carotenoids and gibberellin plant growth regulators, and cell wall biochemistry.

Applicants should have a good research record in the use of recombinant DNA techniques to study either primary plant metabolism or its regulation, photosynthesis, regulation of plant development, signal perception and transduction or organelle biogenesis. The successful candidates will be expected to develop vigorous externally funded research programmes, and applications should include outline proposals for future research.

The College is situated 20 miles outside central London on a wooded campus site near Windsor, Heathrow and the M25.

The appointments, to take effect from October 1989, will be at appropriate points on the Lecturer grade A (£10,910-£16,150) or B (£16,700-£20,960) including London allowance, according to qualifications and experience.

The positions may be discussed informally with Professor J R Bowyer (telephone 0784 34455 ext. 3803). Further details may be obtained from the **Personnel Office, Royal Holloway and Bedford New College, Egham Hill, Egham, Surrey, TW20 0EX**, to whom applications (three copies) including cv, publication list and the names of three referees should be sent not later than April 30th 1989. (8796)A

### INSTITUTE OF CANCER RESEARCH Programmer/Statistician

This post is with the joint Department of Physics of the Institute of Cancer Research and the Royal Marsden Hospital, to assist with research aimed at improving the effectiveness with ultrasound as applied to cancer medicine. It is concerned with the management, processing and statistical analysis of data acquired from the ultrasound images and would be suitable for someone with a background in computer programming, statistics, a physical science, or a related subject. An interest in computer databases and/or expert systems would be useful.

The Physics Section has an active and broad-based research and teaching programme and provides a stimulating environment for someone wishing to work in the bio-medical field. The post will be based at the Sutton Surrey campus. Good sports and social facilities are on-site.

Appointments will be on MRC salary scales for technicians, in the range £7811-£10711 per annum (including London Weighting).

Informal enquiries can be made to Dr. J.C. Bamber on 01-642-6011, ext. 3343.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

To apply, please submit a Curriculum Vitae, in duplicate, with the names and addresses of two referees to **The Personnel Officer, the Institute of Cancer Research, 17a Onslow Gardens, London SW7 3AL** quoting reference number 1.89.T.N.65. (8816)A

### UNIVERSITY OF BIRMINGHAM

Department of Social Medicine

Applications invited for the post of

### RESEARCH ASSOCIATE 1B

to work on an MRC funded project examining the effects of smoking and stopping smoking in pregnancy on the long-term physical and cognitive development of the child. Applicants should have a degree in either single or joint honours psychology and should have used a car. Suitable applicants could register for the degree of PhD.

The appointment is for a three year period from July, August or September 1989. Salary on scale £8,675-£11,680 plus superannuation.

Further particulars and application form available from **Senior Assistant Registrar, Medical School, Birmingham, B15 2TJ** to whom **completed applications (3 copies) should be sent by 17th April 1989**. Quote Ref: RA/SM/CMC.

AN EQUAL OPPORTUNITIES EMPLOYER (8789)A

# MOLECULAR BIOLOGISTS

## DEVELOP THE BIOPHARMACEUTICAL PRODUCTS OF THE 1990s

Celltech is one of Europe's foremost biotechnology companies. From our purpose-built facilities in Slough, we are among the leaders in the research and manufacture of protein-based drug products – the therapeutics for the 1990s and beyond.

We now have a number of opportunities for experienced molecular biologists to join our existing teams in the successful Research Division.

A **Senior Research Scientist** to join our Eukaryotic Molecular Biology Group working on gene expression and heterologous protein secretion. The ideal candidate will be primarily responsible for carrying out innovative research into yeast and mammalian recombinant host systems.

With a PhD in Molecular Biology/Genetics, you will have had up to 3 years' post-doctoral experience of independent research in academia or industry. Ideally, you should have a knowledge of yeast/fungal genetics and also an interest in prokaryotic and mammalian expression. **Ref: 199.**

A **Senior Research Scientist** to join our Mammalian Expression Group working on the expression of recombinant products from mammalian cell lines and making a significant contribution to the development of new products.

Qualified to PhD level, you should have up to 3 years' post-doctoral research experience in Molecular Biology/Molecular Genetics, preferably of eukaryotic cellular functions. You will also have proven ability in conducting independent research and a keen interest

in applying your knowledge as part of a strong team developing novel methods of gene expression. **Ref: 228.**

A **Life Scientist** to join our Eukaryotic Molecular Biology Group engaged in innovative recombinant technology and tissue culture research. You will work on the cloning and expression of recombinant protein in mammalian and microbial host systems, and help to develop our portfolio of biopharmaceutical products.

You should have a good honours degree in a Biological Science, with emphasis on molecular and cell biology. With up to 3 years' academic or industrial laboratory experience, you will have developed a knowledge of animal cell culture and preferably recombinant DNA technology. **Ref: 262.**

Celltech is a young company: attracting and retaining the very best people has been vital to our success, and will always be a key priority. Whether working on your own initiative or as part of a multi-disciplinary team, you'll find that good interpersonal skills are essential in our fast-moving and results-oriented environment.

You can be sure your potential will be recognised and valued – our scientists enjoy competitive salaries and excellent career opportunities, supported by a comprehensive benefits package and relocation assistance where appropriate.

Please write with full details of your experience to date and quoting the relevant reference number to Mrs Jane Smith, BSc C BIOL MI BIOL, at Celltech, 216 Bath Road, Slough, Berkshire SL1 4EN.





## ENDOCRINOLOGY DIVISION

### Cell Biologist

We seek a post-doctoral scientist to develop cellular aspects of endocrinology applied to the characterization and standardization of hormones, including those derived from modern biotechnologies, and associated research work. The appointment will be initially for five years with the possibility of tenure at the end of that period.

The Successful Candidate will be expected to establish endocrine cell lines and study the actions of hormones, growth factors and other protein modulators. Experience in cell culture and molecular aspects of peptide hormones will be an advantage.

The Institute is located in newly-built premises in Hertfordshire, within reach of St Albans and central London and offers excellent facilities for modern biological and molecular sciences.

Salary will be on Medical Research Council scales, dependant on age, qualifications and experience.

*Informal enquiries may be made to Dr S L Jeffcoate, ext 227.*

To apply please submit a full C.V. together with the names and addresses of three referees to: **The Personnel Office, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3GQ.** Telephone 0707 54753 Ext 458. Please quote reference HO/047A.

**Closing date for applications: 14th April 1989.**

**NIBSC**

National Institute for Biological Standards and Control  
(8811)A

## Pharmacology of Shock & Inflammation

### Merck Sharp & Dohme Research Laboratories

MSDRL is recognized as the number one pharmaceutical company in the world, with an outstanding reputation for basic research and its application to the discovery and development of safe and effective medicines.

The Department of Cellular and Molecular Pharmacology of Merck Sharp & Dohme Research Laboratories is engaged in multi-disciplinary studies aimed at developing novel therapeutic strategies for the treatment of inflammatory diseases. As part of our expanding research program, we seek an experienced Ph.D. Scientist with broad-based expertise in the measurement of cardiovascular and/or pulmonary function in animal (dogs, swine, sheep, non-human primates) models of shock and inflammation.

Qualified candidates will be creative and enthusiastic scientists with relevant post-doctoral research experience, a sound publication record and excellent communication skills.

Merck Sharp & Dohme Research Laboratories are located in Rahway, New Jersey approximately 25 miles from New York City. Our salaries, benefits and growth potential are excellent. Interested candidates should submit their curriculum vitae with the names of three references and salary requirements to: **Ms. Lonnie L. Deetz, R80M-TL1, Merck & Co., Inc., P.O. Box 2000, Rahway, NJ 07065.**

AN EQUAL OPPORTUNITY EMPLOYER

(NW3506)A

**Merck & Co., Inc.**



## THE UNIVERSITY OF ZAMBIA

Applications are invited from suitably qualified candidates to fill the following posts:

### SCHOOL OF MEDICINE

#### DEPARTMENT OF ANATOMY

#### Associate Professor/Senior Lecturer/Lecturer (3 posts)

Applicants should have a higher degree (M.Sc., Ph.D or equivalent) in morphological Science. A medical degree (M.B., Ch.B., M.D. or equivalent) is desirable but not essential for applicants with experience in teaching and research in human anatomy in a Medical or Dental School. Duties will involve teaching medical undergraduate and postgraduate students and various cadres of paramedicals (post-basic nursing, physiotherapy etc.). The human anatomy course is divided into four subcourses: Gross Anatomy, Histology, Embryology and Neuroanatomy, and the successful applicant will be required to teach at least two of these fields.

### SCHOOL OF NATURAL SCIENCES

#### DEPARTMENT OF BIOLOGY

#### Senior Lecturers/Lecturers in the following areas:

Molecular Biologists with interest in Cell Ultrastructure, Protein Structure and Function, Biomembranes and Bioenergetics.

Animal Physiologist with interest in reproductive biology.

Entomologist with interest in vector biology of parasites.

Geneticist specialising in cytogenetics with interest in population genetics.

Tracheophyte Biologist able to teach taxonomy, morphology and anatomy of angiosperms and gymnosperms.

Applicants are required to lecture to second up to fourth year students and should have a Ph.D with 2-3 years post-doctoral research experience, preferably University level teaching and a demonstrated interest in research.

### SCHOOL OF AGRICULTURAL SCIENCES

#### Professor/Senior Lecturer/Lecturer in Farm Management

Applicants should have an M.Sc or Ph.D degree in Agricultural Economics with emphasis on Farm Management. Extensive teaching and research experience at University level in a developing country preferred. Duties will include conducting lectures and tutorials in Production Economics, Farm Accounts, Farm Budgeting and Planning, and Farming Systems to undergraduate students; supervising practical training of students; initiating research or continuing on-going research into management problems of Peasant and Commercial farms in Zambia.

#### Professor/Senior Lecturer/Lecturer in Extension Education/Rural Sociology

Candidates should have an M.Sc or Ph.D degree in Agricultural Extension Education/Rural Sociology with extensive teaching experience at the University level. Research and extensive experience in Africa/developing countries would be preferred. The appointee will teach and conduct tutorials in Agricultural Extension/Rural Sociology undergraduate courses; supervise practical training of students; and carry out socio-economic research related to the needs of Zambia.

Salaries: Entry point and salary will depend on qualifications and experience in the range of (per annum): Professor ZMK 50,652-55,260; Associate Professor ZMK 42,600-48,648; Senior Lecturer ZMK 36,204-44,844; Lecturer Grade I ZMK 32,604-37,836; Lecturer Grade II ZMK 27,252-31,860; Lecturer Grade III ZMK 24,348-26,364.

All posts are tenable immediately and the University offers contracts of up to four years (renewable). Other benefits include family passages; annual leave; children's education allowance and holiday passages; non-practising allowance for medical personnel etc.

Detailed applications with full curriculum vitae, and three names and addresses of referees should reach the Registrar, University of Zambia, P.O. Box 32379, Lusaka, Zambia, not later than 10 April 1989. (W5996)A

**nature**

the widest international selection of jobs  
in science — EVERY WEEK



## UCLA CELL AND MOLECULAR PHYSIOLOGY

The Department of Physiology invites applications for tenure track faculty positions at the level of

# Assistant Professor

Outstanding candidates working in any area of contemporary physiology will be considered, but those in smooth muscle, reproduction and development, endocrinology, sensory transduction, and the cardiovascular system are encouraged to apply. Successful candidates are expected to have a Ph.D. or M.D. with post-doctoral experience, to provide evidence that they can pursue a vigorous, independent research program and to participate in our teaching program.

Resources and an excellent environment exists for developing an academic career within an interactive community. Interested scientists should send their curriculum vitae, a statement of present and future research interest, and names of three references to:

**Ernest M. Wright, D.Sc.,  
Professor and Chairman,  
Department of Physiology,  
UCLA School of Medicine,  
Los Angeles, CA 90024-1751.**

The University of California is an Equal Opportunity Affirmative Action Employer.

(NW3500)A

### UNIVERSITY OF BRISTOL DEPARTMENT OF PHYSIOLOGY POSTDOCTORAL RESEARCH FELLOW IN SENSORY NEUROPHYSIOLOGY

Applications are invited for a three-year M.R.C.-funded post, starting on July 1st or as soon as possible thereafter, to study the immunocytochemical and electrophysiological properties as well as the ion channels of primary sensory neurones with known sensory receptor properties. Intracellular recordings will be made in an *in vitro* skin/nerve/ganglion preparation. Experience in electrophysiology an advantage. Salary on Grade 1A scale.

Informal enquiries can be made by telephoning Dr Sally Lawson on 0272 303463.

**For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference A287.**

*An Equal Opportunities Employer* (8790)A

### PROFESSORSHIP OF MICROBIOLOGY

Applications are invited for the above vacant office in the Department of Food Microbiology.

Salary scale: IRE30,747-IRE35,567 p.a.

Application forms and further details may be obtained from the undersigned. Latest date for receipt of completed applications is **Friday, 28 April, 1989.**

M.F. Kelleher  
Secretary

(8828)A

Coláiste na hOllscoile Corcaigh  
University College Cork



### JOHN INNES INSTITUTE **Seeds**

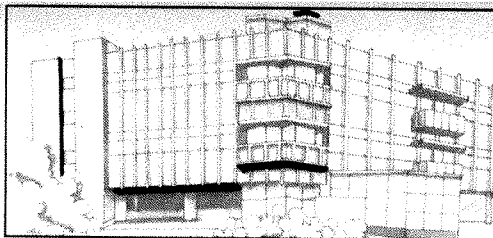
## POSTDOCTORAL RESEARCH APPOINTMENT IN MOLECULAR PLANT VIROLOGY

Applications are invited for a postdoctoral research scientist for a project funded by ICI Seeds in the Department of Virus Research of the John Innes Institute. The project concerns the isolation and molecular characterization of sugar beet viruses. Experience in molecular biology is desirable, but not essential. The successful applicant will join an active research group working on various aspects of plant virus molecular biology. The appointment will be for two years duration. Further particulars may be obtained from Dr R Hull at the John Innes Institute.

The appointment will be on the University R & A 1A scale and the salary in the range £9,865 to £15,720. Contributory superannuation scheme. Equal Opportunities Employer.

Applications with full particulars, CV, and the names of two referees should be sent to the **Personnel Officer, John Innes Institute, Colney Lane, Norwich, NR4 7UH** to arrive not later than 24 April 1989. Quote reference VIR/348.

*The John Innes Institute is associated with the AFRC  
Institute of Plant Science Research.* (8822)A



## MERRELL DOW RESEARCH INSTITUTE STRASBOURG CENTER

### Postdoctoral behavioural pharmacologist

We have an opening for a postdoctoral (Ph.D) behavioural scientist to establish animal models of learning and memory as part of a program aimed at the development of novel remedies for cognitive disorders. Prior experience of testing cognitive function in animals is essential for this post. The position is available for a period of 1-2 years. The Merrell Dow Research Institute has a liberal publication policy. Knowledge of French is desirable, but not a requirement for this position. Interested applicants should send a curriculum vitae and names of three references to:

Mrs M.J. BILDSTEIN, Personnel Manager Merrell Dow Research Institute, 16 rue d'Ankara,  
F-67084 STRASBOURG Cedex.

(W5984)A

## UNIVERSITY OF DUNDEE DEPARTMENT OF BIOLOGICAL SCIENCES POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited to fill the above AFRC-supported position to work with Professor John Raven and Dr Janet Sprent on the role of soil-nodule exchanges in the iron, molybdenum, acid-base and water economy of  $N_2$ -fixing legumes.

Salary on the Research Assistant 1A scale (£9,865-£15,720). Initial salary (age 27+) will be at £11,680.

The position is available for three years from 1 May 1989 or as soon thereafter as can be arranged.

Applications in writing, including a full CV (2 copies) and the names of two referees, as soon as possible to, the **Personnel Office, The University, Dundee, DD1 4HN**. Please quote ref: EST/410/89/N.

Informal enquiries to Professor Raven, University of Dundee, Tel. (0382) 23181 Ext. 4281, from whom further particulars can be obtained. (8805)A

MEDIA SYSTEM



KAROLINSKA  
INSTITUTE  
STOCKHOLM,  
SWEDEN  
DEPARTMENT OF  
CLINICAL GENETICS



### Postdoctoral research assistant in molecular genetics

Applications are invited from PhD graduates with a background in molecular genetics for a postdoctoral research position on any of the following projects for a minimum of 12 months:

1. Molecular endocrinology; gene structure and function in Non-Insulin-Dependent-Diabetes, 21-Hydroxylase Deficiency, and of the human growth hormone independent binding protein for insulin-like growth factors
2. Genetic toxicology; molecular mechanisms of spontaneous and induced mutations in human somatic cells
3. Cancer genetics; molecular and chromosomal mechanisms of human cancer and inherited predisposition to neoplasia

Applications including Curriculum Vitae and names and addresses of two referees should be sent to:

**Professor Jan Lindsten,**  
Department of Clinical Genetics,  
Karolinska Hospital,  
S-10401 Stockholm, Sweden.

tel +46-8-729 2469; fax +46-8-32 77 34

(W6002)A

## RESEARCH SCIENTIST MOLECULAR BIOLOGIST: MOLECULAR GENETICIST

A six-year tenure track position is available in the ICRF Laboratory of Molecular Pharmacology in Edinburgh. The overall aims of the group are to understand the factors which determine individual susceptibility to cancer as well as molecular mechanisms of tumour cell resistance to cytotoxic drugs. Candidates should have a strong background in eukaryotic or prokaryotic molecular biology and/or molecular genetics and will be expected to work independently on themes complementary to the main aims of the group.

The ICRF group is situated in the University of Edinburgh Department of Biochemistry and works closely together with other Departments in the University. The successful candidate will be considered for an honorary position within the University.

Salary range: £14,500-£23,000

For further information contact Dr. C. R. Wolf, I.C.R.F. Lab of Molecular Pharmacology, Hugh Robson Building, George Sq., Edinburgh EH8 9XD, U.K. Tel: 031-668-3343.

**Applications should be made by sending a full curriculum vitae together with the names and addresses of three referees to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX quoting reference 78/R.**

Closing date for applications: 21st April 1989

Smoking is actively discouraged.

(8834)A

**I M P E R I A L  
CANCER RESEARCH FUND**

## UNIVERSITY OF ST ANDREWS DEPARTMENT OF PSYCHOLOGY

Applications are invited for the post of

### RESEARCH TECHNICIAN GRADE 5

required for international joint research prospect on "Temporal lobe mechanism in Memory" supported by Japanese NEDO. The project will focus on visual neurones responsive to meaningful stimuli (faces and actions).

The post is available from April 1st for one year in the first instance (renewable for two years) at a starting salary of £8,088 per annum. Applicants should have HNC or equivalent, together with experience in either biological science or electronics/computing.

The appointee will be involved in electrophysiological data collection. Enquiries and applications with a full curriculum vitae and names of two referees familiar with the applicants work should be sent to **Dr D Perrett, Psychology Department, University of St Andrews, St Andrews, fife, KY16 9JU** as soon as possible. (8808)A

## MATHEMATICAL GEOLOGIST

The sandstone cementation, cation transport, heat and fluid movement in the 360 Myr old sedimentary basin of N. England is being researched by three Ph.D students. A central part of the project is to quantify mass transfer of minerals, heat and oil by **developing computer models** of ancient compactional fluid flow or diffusion through subsiding porous rock in this basin and the North Sea. We need a strong mathematical or computing background with a Ph.D, or a BSc. plus relevant experience in porous media fluid flows. Based at Glasgow University, with excellent VAX and SUN computing in Dept. Salary 3yrs on 1A scale starting £11,015. Apply with CV and three referees by 4 weeks after publication date to **Dr. R S Haszeldine, Applied Geology, Glasgow G1 1XJ, Scotland. Tel 041-552-4400 ext 2148.** (8823)A



**SOUTHAMPTON GENERAL  
HOSPITAL**

WESSEX REGIONAL  
IMMUNOLOGY SERVICE

**SENIOR SCIENTIFIC  
OFFICER**

To take charge of monoclonal antibody production, standardisation and use for immunophenotyping of patients with haematological neoplasms and immunodeficiency disorders. Research activities and industrial liaison encouraged. Post would suit recent Ph.D.

Salary: £11,778-£15,394.

For informal visits contact Dr. J.L. Smith on (0703) 777222 Ext. 3490.

Application form and job description from Unit Personnel, Level C, Centre Block, Southampton General Hospital, Southampton, SO9 4XY. Tel. (0703) 777222 Ext. 4100.

Closing date: 6.4.89.

Working Towards Equal Opportunities. (8806)A

**UNIVERSITY OF  
MANCHESTER  
DEPARTMENT OF CHEMISTRY  
POST-DOCTORAL RESEARCH  
ASSOCIATE IN ORGANIC  
SYNTHESIS**

Applications are invited for this SERC funded post to work with Professor E. J. Thomas on the total synthesis of a group of biologically active marine natural products. Initial salary range £9,865 - £11,680 p.a. Superannuation.

Appointment will be for one year in the first instance, starting on October 1st, 1989.

Applications including a c.v. and the names and addresses of two referees should be sent as quickly as possible to **Professor E. J. Thomas, Department of Chemistry, the University, Manchester M13 9PT** from whom further particulars may be obtained.

*The University is an equal opportunities employer.* (8793)A

**MOLECULAR BIOLOGIST**

Two Postdoctoral Positions — Nucleic acids experience required. (1) Work with gene transfer, regulated expression, *cis* and *trans*-acting factors regulating genes for steroidogenic enzymes. (2) Work with bacterial and yeast expression systems, maximizing expression, purifying and characterizing expressed products.

Send CV and letters of reference to **Dr. Walter L. Miller, Laboratory of Molecular Biology, Room 677-S, University of California, San Francisco 94143-0434.** (NW3498)A

**COLLEGE OF SCIENCE  
KUWAIT UNIVERSITY  
STATE OF KUWAIT**

The Kuwait University College of Science, Dept. of Botany and Microbiology invites applications for posts of Professor, Associate Professor and Assistant Professor for the academic year 1989/1990, tenable February 10, 1990 in the following disciplines:

**Plant Breeding  
Microbial Physiology  
Agriculture & Economic Botany**

The language of instruction of the College of Science is English. The method of teaching is based on the credit hour system. Applicants must hold the Ph.D. or its equivalent at the time of application. For applications and conditions of service write to:

**Kuwait University Office  
3500 International Dr., N.W.  
Washington, D.C. 20008  
(202-363-8055)**

All applications, together with non-returnable copies of academic qualifications and representative publications should be sent by registered post directly to:

**Dean  
College of Science  
P.O. Box 5969  
13060 Safat  
Kuwait**

All applications must be received by August 31, 1989.

(NW3510)A

**The Elmer V. McCollum Professor  
and  
Chair  
Department of Biochemistry  
The Johns Hopkins University  
School of Hygiene and Public Health**

Applications and nominations are invited for the position of Chairperson of the Department of Biochemistry at the Johns Hopkins School of Hygiene and Public Health. The general research interests of the current faculty include: Biochemistry — enzyme structure and mechanisms; peptide chemistry; chromatin and nucleic acid chemistry; mechanisms of nucleic acid replication, recombination and repair; structure and function of cell surface molecules; somatic mutation and antibody diversity; cell metabolism; influence of nutrition in carcinogenesis. Biophysics — structure and interaction of biopolymers in cells; synthesis of oligo- and polynucleotides of designed structure and sequence; structure and function of the mammalian genetic apparatus; development of antiviral and chemotherapeutic agents; basic mechanisms of aging. The Department also participates in a University-wide concerted effort in areas of macromolecular assemblies and interactions for which it has received considerable Federal and private support.

Candidates must have an outstanding record of research achievement in any area of biochemistry, biophysics or molecular biology, whether or not the area is currently represented in the Department; and the desire and ability to provide strong leadership to an already excellent, vibrant, growing department in the interdisciplinary setting of the School of Hygiene and Public Health.

Interested persons are encouraged to submit a curriculum vitae to:

**Dr. Barry Zirkin  
Chairman of the Biochemistry Search Committee  
Division of Reproductive Biology  
The Johns Hopkins School of Hygiene and Public Health  
615 North Wolfe Street  
Baltimore, MD 21205**

The Johns Hopkins University is an Equal Opportunity employer

(NW3504)A

## AGRICULTURAL AND FOOD RESEARCH COUNCIL (AFRC)

# HEAD OF POLICY, PLANNING AND ASSESSMENT

Swindon

up to £36,700

The AFRC undertakes basic and strategic research underpinning the agricultural, food and emerging biotechnological industries of the UK. Research is carried out in institutes, universities and other HEIs. The AFRC has 4,700 staff and an annual budget of £135m.

The Head of Policy, Planning and Assessment will lead a team helping to shape future development of the Council's scientific activities. These relate, for example, to environmental issues, novel applications of genetic expression in plants and animals, food quality and safety; and the implications for British research of the single European market. The person appointed will have a central role in the Council's planning activities, including responsibility for the annual AFRC Corporate Plan. The post also carries responsibility for developing procedures for the Council's management of research, including monitoring and assessment through performance indicators and the development of management information systems. Policy analysis and briefing for the Secretary

of the Council are an important continuous activity.

Candidates should have a background in scientific research, preferably in the biological sciences, and an understanding of the administrative machinery of government and the Research Councils. Experience in quantitative analysis and in economic assessment would be an advantage. Strong qualities of oral and written communication are essential.

Starting salary will be on a scale between £28,170 and £31,602. Performance-related increments are attainable, with further increases to £36,786. Benefits are similar to those in the Civil Service.

Please apply in writing for an application form to: Mr R J Davies, Manager, Central Office, Agricultural and Food Research Council, Wiltshire Court, Farnsby Street, Swindon, Wilts SN1 5AT.

Closing date for receipt of completed application forms: 14th April 1989.

The AFRC is an equal opportunities employer.

(8832)A



## UNIVERSITY OF OXFORD NUFFIELD DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY

John Radcliffe Hospital  
Headington OXFORD OX3 9DU

### Postdoctoral Research Assistant

Applications are invited for a postdoctoral position to work on gene abnormalities and transcription in human breast cancer. The person appointed should have experience in molecular biology. The successful applicant will work in the relatively new group of about 10 people who are investigating the molecular pathology of "early" human breast cancer.

The salary is funded by the Cancer Research Campaign for 3 years in the range of £8,675 - £13,365.

Applications, with the names of referees, should be sent to Professor J. O'D McGhee at the above address by 13.4.89. He will provide detailed particulars of the specific problem being investigated by this Group.

Oxford University is an equal opportunity employer. (8820)A

## The Faculty of Medicine, University of Geneva has an opening for a

### FULL PROFESSOR IN ITS DEPARTMENT OF PATHOLOGY

This is a full time post. The successful candidate will be the Chairman of the Department of Pathology and Chief of the Institute of Hospital Pathology; the post involves the responsibility for teaching at the pre- and post-graduate level; supervision of seminars and of clinical research.

Qualification required: M.D.

Board qualified (or equivalent)

Stating date: 1st of October 1989 or by arrangement.

Applications and CV should be sent until 30 May to the

**Secrétariat de la Faculté de Médecine**

**Centre Médical Universitaire**

**1 rue Michel-Servet, 1211 Genève 4, Switzerland**

**Phone 022/22.91.82**

where further particulars of the post can be obtained.

(W5990)A

### TENURE-TRACK POSITION

available for Ph.D. or M.D. at assistant or associate professor rank to evaluate cell-mediated immune responses to HIV infections and vaccines. Candidates will be expected to develop vigorous research program, with opportunity to participate in evaluations of other viral and parasitic vaccines under investigation at the Center for Immunization Research.

Primary appointment is in the Department of Immunology and Infectious Diseases in School of Public Health; option for joint appointment in Division of Infectious Diseases in School of Medicine.

Please submit C.V. and names of 3 references to

**Dr. Noel Rose c/o Dr. M.L. Clements,**

**Johns Hopkins University**

**(JHU) Center for Immunization Research,**

**624 North Broadway, Baltimore, MD 21205.**

JHU is an Equal Opportunity/Affirmative Action Employer.

(NW3496)A

## AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY, INC.

### EDITOR OF CLINICAL CHEMISTRY

The American Association for Clinical Chemistry, Inc., invites nominations for position of Editor of *Clinical Chemistry*, the world's leading journal in clinical laboratory science with a circulation of 15,000 and one of the 100 journals most frequently cited.

Candidates must hold the Ph.D. or M.D. degree and have broad knowledge of clinical chemistry and extensive experience in scientific and medical writing. The candidate should be familiar with experimental design and be able to judge the scientific validity and importance of research reports and review articles. The successful candidate will have a strong scientific record and the desire to expand and extend the scope of clinical chemistry. The candidates will have the decision making skills associated with publishing and the ability to interact constructively with the scientific community.

Both full and part-time candidates will be considered. Interested individuals should send a letter of application, a curriculum vitae, reprints of two of their publications and the names of five individuals who could serve as references by June 15, 1989, to:

**Theodore Peters, Jr., Ph.D., Chairman,  
Search Committee for Editor, Clinical Chemistry,  
American Association for Clinical Chemistry, Inc.,  
2029 K Street, N.W., Seventh Floor,  
Washington, DC 20006**

(NW3505)A

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Fax 03-267-8746

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# PROTEIN CHEMIST



Hoffmann-La Roche has a unique opportunity for a **Senior Scientist** in protein chemistry to join our Molecular Structure Group and perform research leading to new pharmaceutical agents based on the three-dimensional structures of proteins as targets or templates.

You will establish and lead a protein chemistry laboratory which will purify proteins for structural determinations via crystallography and NMR spectroscopy; perform functional and mechanistic studies on the proteins; and collaborate in drug design based on this structural and functional information. Proteins to be investigated include enzymes, effectors and receptors (for example, HIV proteins).

Qualifications include a PhD in Chemistry or Biochemistry, and experience in purifying proteins using modern chromatographic methods. A strong interest in the three-dimensional structure of proteins, post-doctoral experience and knowledge of enzymology, genetic engineering, and protein expression are highly desirable.

We offer a comprehensive compensation package, and a state-of-the-art scientific environment that encourages innovation and professional growth. For consideration, please send your resume, indicating present salary, to: **Eleanor M. Malone, Department VM, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.** We are an equal opportunity employer.



**Hoffmann-La Roche**

Working Today For A Healthier Tomorrow. (NW3503)A

## POST-DOCTORAL POSITION MOLECULAR IMMUNOLOGY MT. SINAI SCHOOL OF MEDICINE

A position is available to study molecular mechanisms regulating MHC gene expression following T cell activation. The relationship between MHC antigen expression and T cell function will also be studied. Training in molecular biology is desirable. Prior exposure to cellular immunology/immunogenetics would be an asset, but the position is also appropriate for someone desiring experience in these areas.

Send curriculum vitae and the names and addresses of three references to:

**Karen S. Zier, Ph.D.**  
**Mt. Sinai School of Medicine**  
**Division of Molecular Medicine**  
**Box 1055**  
**One Gustave L. Levy Place**  
**New York, NY 10029**

(NW3494)A

## NATIONAL INSTITUTE FOR MEDICAL RESEARCH GENES AND CELLULAR CONTROLS GROUP

### SHORT-TERM SCIENTIFIC STAFF POSITIONS

Applications are invited for two three year appointments to the Scientific Staff, tenable in the Laboratory of Eukaryotic Molecular Genetics (Head: Dr P W J Rigby). The research, which will be under the supervision of Dr A I Magee and Dr R S Buxton, will involve studies of the molecular biology of cell adhesion molecules associated with an intercellular junction, the desmosome. One project, that supervised by Dr Magee, (Job reference 5425/GEKG) will involve protein purification and sequencing, cell culture and the expression of cloned genes, with a view to understanding protein function. The other, to be supervised by Dr Buxton (Job reference 5311/GEKG) will involve the use of a wide range of recombinant DNA techniques to study the structure and function of cloned genes encoding desmosomal proteins. Experience in relevant techniques would be advantageous but is not essential as full training can be given in all of them.

Job reference 5311/GEKG is available immediately, the other from October 1989.

Salary is in the range £11,070-£14,500 + £1,650 London Allowance per annum.

Informal enquiries may be made by telephoning Dr Magee or Dr Buxton on 01-959 3666 extension 2224 or 2225 respectively.

Applications, including a curriculum vitae, and names of two professional referees, should be sent to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London, NW7 1AA** quoting the relevant job reference. Closing date for this post will be 21st April 1989.

An Equal Opportunities Employer. (8818)A

**MRC**

## UNIVERSITY OF ULM



Applications are invited for the position of an

### university assistant

at the Department for Virology. The position is available for approx. 5 years.

The candidate is expected to head a small research group and has the chance to cooperate in the Sonderforschungsbereich 322 "Lymphohemopoiesis". There is a possibility for habilitation. His/her research should center around molecular problems of viral gene regulation, cell tropism or antiviral

immunity.

Applications incl. CV, list of publications, research proposal and two letters of reference by April 30, 1989, at the latest, should be directed to

**Prof. U. Koszinowski**  
**Department of Virology**  
**University of Ulm**  
**Albert-Einstein-Allee 11**  
**7900 Ulm**  
**West Germany**

(W5989)A





**ROYAL HOLLOWAY AND BEDFORD  
NEW COLLEGE**  
University of London

**DEPARTMENT OF GEOLOGY  
RESEARCH STUDENTSHIP  
IN STABLE ISOTOPE GEOCHEMISTRY**

A 3 year studentship, supported by V G Isogas Ltd, is available for research into the stable isotope geochemistry of fluid processes in the mantle and lower crust. Fluid inclusion geochemistry will be investigated using state-of-the-art gas extraction and mass spectrometric techniques.

The student will be based in the new stable isotope laboratory at RHBNC and will also have opportunities to utilise research and developmental facilities at V G Isogas Ltd. Attendance at international conferences will be encouraged.

The project will be jointly supervised by Dr D P Matthey (RHBNC) and Dr R A Exley (V G Isogas).

Applications, including a brief CV and names of two referees, should be sent to: **Dr D P Matthey, Department of Geology, RHBNC, University of London, Egham Hill, Egham, Surrey, TW20 0EX.** To obtain further information please telephone Egham (0784) 34455 ext. 3587. (8798)A

**BRITISH MEDICAL JOURNAL  
ASSISTANT EDITOR**

The BMJ seeks a new assistant editor with at least five years' experience of medical or scientific publishing. The applicant need not be medically qualified, although a qualification would be welcomed. The successful applicant will join the editorial team and will participate in selecting scientific articles and commissioning and writing articles. A broad understanding of medico-politics will be welcome as will experience of management. The journal's three senior editors are all retiring within the next two years, so opportunities for greater responsibility may arise.

**The salary will be not less than £24,000** and the starting date will be negotiable.

Applicants should send full particulars of qualifications and experience, together with the names and addresses of three people to whom reference may be made, to

**The Editor BMJ  
BMA House, Tavistock Square,  
London WC1H 9JP**  
not later than 21 April. (8819)A



**FELLOWSHIPS**

**COLUMBIA UNIVERSITY  
POSTDOCTORAL FELLOW IN  
MOLECULAR ENDOCRINOLOGY**

An NIH-funded position in the Department of Biochemistry and Molecular Biophysics is available July '89 for studies of intracellular pathways of insulin, growth factor, and proto-oncogene actions in fibroblasts. The projects use both protein biochemistry and molecular biology approaches. Experience in the biochemistry of polypeptide hormone response pathways or in molecular biology techniques desirable. US citizenship or residency required. May participate in Endocrinology and Metabolism Clinical Fellowship if an M.D. and qualified. Send curriculum vitae and names/phone numbers of three references to Dr. R.W. Rees-Jones, Department of Medicine, Columbia University, 630 W. 168th St., NY, NY 10032. *Columbia University takes Affirmative Action to ensure Equal Opportunity.* (NW3508)E

**California Institute of Technology  
The Texaco Postdoctorate Fellowship**

The California Institute of Technology announces a fellowship award from a fund endowed by the TEXACO PHILANTHROPIC FOUNDATION. This fellowship carries an annual stipend of \$32,000 from Fall 1989. Travel costs to Caltech will be covered by a supplement of up to \$1,000. The duration of the appointment will normally be one-two years.

This fellowship program has been established to support the research of scientists, typically within two years of receipt of the Ph.D. It is the intent of this program to identify and support innovative and creative work in the earth sciences, with particular emphasis on interdisciplinary work. Applicants with training in physics, chemistry, biology or computer sciences are urged to apply. The Caltech faculty is currently active in Atmospheric and Planetary Sciences, Geochemistry, Petrology, Geology and Geophysics.

Application forms may be obtained from writing to  
**Professor G. J. Wasserburg, Chairman,  
Division of Geological and Planetary Sciences,  
Mail Code 170-25 (TPF),  
California Institute of Technology,  
Pasadena, California 91125.**

**COMPLETED APPLICATIONS WITH REFERENCES  
SHOULD ARRIVE AT CALTECH BEFORE 31 MAY, 1989.**

Fellowship candidates will automatically be considered for other available postdoctoral positions at Caltech in their fields of interest.

Caltech is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.

(NW3499)E

**Postdoctoral  
Fellow Program**



**Biochemistry  
of Plant Lipids  
and Starches**

Applications are invited for Postdoctoral Fellowships to join a dynamic and ongoing basic research program in plant biochemistry and molecular biology. Project includes the purification and characterization of enzymes involved in starch and lipid synthesis, and the introduction and characterization of heterologous genes in oil rapeseed and potato.

Highly motivated individuals with a strong background in biochemistry and molecular biology should apply by sending a CV and the name of three references to: Calgene Fellow Program, File #782N, 1920 Fifth Street, Davis, CA 95616. An equal opportunity employer.



(NW3511)E

**CALGENE**

## MOLECULAR GENETICS/COLUMBIA UNIVERSITY POSTDOCTORAL FELLOWSHIPS

We invite inquiries from candidates with a PhD or MD degree for a two year (minimum) postdoctoral fellowship to study heritable neurological and psychiatric disorders. Two awards beginning at \$30,000 per annum are available, one immediately and one in July 1989. Preference will be given to candidates with postdoctoral experience or outstanding thesis work including chromosomal mapping and cloning, or a related area of molecular biology. Projects include mapping and cloning of disease gene regions; development of brain specific DNA markers; and computer analysis of complex banding patterns. Positions are supported by the W.M. Keck Foundation.

Please address inquiries plus curriculum vitae to: **Dr. Conrad Gillian, Molecular Genetics Laboratory, College of Physicians and Surgeons at Columbia University, Box 23, 722 West 168th St., New York, N.Y. 10032.**

EO/AA employer.

(NW3509)E

## BRITISH DIABETIC ASSOCIATION SENIOR RESEARCH FELLOWSHIP

Applications are invited from post-doctoral workers with substantial experience and achievement in diabetes research for a Senior Fellowship.

The Fellowship will provide the salary of the holder on University scales 1A or 2 and recurrent expenditure of up to £5,000 per annum. It will be tenable for 5 years in the first instance, renewable subject to satisfactory review up to a maximum of 10 years.

The closing date for applications is 8th May 1989. These should be sent to **Dr M Murphy, British Diabetic Association, 10 Queen Anne Street, London W1M 0BD.** Tel. 01-323-1531 from whom further information is available if required. (8804)E

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## UNIVERSITY OF EDINBURGH DEPARTMENT OF SURGERY POST DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a post doctoral research post in the above department funded by the Cancer Research Campaign.

The project involves assessing the potential role of lymphokine activated lymphocytes in the treatment of human bladder cancer. Applicants should have previous experience of immunology and tissue culture.

The post, which is funded for 3 years, is on the 1A scale for Research and Analogous Staff, the commencing salary being up to £11,680 per annum.

Further details of the post are available from Mr A.W.S. Ritchie (031-668-3978) or Dr K. James (031-667-1011). Applications with curriculum vitae and names of 2 referees should be sent to **Dr K. James, Department of Surgery, Medical School, Teviot Place, Edinburgh.** Ref No 5653.

Closing date for applications 30th April 1989. (8830)E

## STUDENTSHIPS

## MRC PROTEIN PHOSPHORYLATION GROUP BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF DUNDEE POSTDOCTORAL POSITIONS AND POSTGRADUATE STUDENTSHIPS

Following expansion of the research programme by the Medical Research Council, several postdoctoral positions have become available to work with Professor Philip Cohen and Dr Tricia Cohen. The work is interdisciplinary and applications are invited from candidates with experience in any of the following areas; molecular genetics, protein chemistry, enzymology, molecular pharmacology and immunocytochemistry. A major aspect of the research is concerned with the structure and regulation of protein phosphatases. Recent work within the Group has demonstrated that these enzymes play key roles in cell division and in tumour suppression, and several new phosphatase genes have been isolated. Novel mechanisms for controlling their activities have also been identified that involve interaction with specific targetting subunits. The positions are available from October 1989, but candidates wishing to start at any time up to Spring 1990 will be considered. The appointments are for three or five years in the first instance and will be made at an appropriate point on the University Grade 1A scale, depending on qualifications and experience. Applications are also invited for MRC and SERC postgraduate studentships, which will be supplemented in the first year by £250 (£500 for candidates obtaining a 1st class degree).

The Department is one of the most highly funded in Britain and offers outstanding research facilities and working environment. Housing and accommodation costs are among the lowest in the UK and access to outdoor recreational facilities is unrivalled.

Applications containing a full curriculum vitae and the names and addresses of three referees should be sent to **Professor Philip Cohen, FRS, FRSE, Department of Biochemistry, The University, Dundee DD1 4HN, Tayside, Scotland** as soon as possible. Informal enquiries by telephone (0382-23181) to either Philip Cohen (ext 4238, 4241) or Tricia Cohen (ext 4240). (8824)F

## Coleg Prifysgol Cymru **Aberystwyth** The University College of Wales INSTITUTE OF EARTH STUDIES

Applications are invited for College funded Research Studentships in the following fields:—

- Development of optical dating methods.
- Calcareous microfossils as geo-thermometers.
- Rare elements in fossil invertebrate skeletons.
- Pliocene global warming.
- Cretaceous anoxic events.
- Fluid movement in deforming sediments.
- Historic farming systems and the environment.
- Information technology and rural development.

It is expected that at least four awards will be made to those who have or expect to obtain good Honours degrees in appropriate disciplines. Further details may be obtained from Professor John Lewis, Institute of Earth Studies, University College of Wales, Aberystwyth, SY23 3DB. (Tel 0970 623111 Ext 3345). (8812)F

## UNIVERSITY OF ST ANDREWS DEPARTMENT OF BIOLOGY & PRECLINICAL MEDICINE RESEARCH STUDENTSHIP

Applications are invited for a 3-year SERC Earmarked Research Studentship to work with Professor I.A. Johnston at the Gatty Marine Laboratory on a project to investigate the power output and metabolic cost of muscle contraction in fish under conditions relevant to swimming. Candidates should have, or expect to obtain, a First or 2:1 honours degree in biology or physiology. Letters of application enclosing a full Curriculum vitae, which includes the names of two academic referees, should be sent to **Professor I.A. Johnston, Department of Biology & Preclinical Medicine, Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife KY16 8LB, Scotland**, from whom further particulars are also available.

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(8807)F

# UNIVERSITY OF LIVERPOOL

# SCHOOL OF LIFE SCIENCES

## POSTGRADUATE STUDENTSHIPS

### in the FACULTY OF SCIENCE

The three Departments are Biochemistry, Genetics & Microbiology and Environmental & Evolutionary Biology. The latter includes the Marine Biological Laboratory, Port Erin, Isle of Man. Studentships will be available for research leading to a Ph.D. in the following areas:

#### Department of Biochemistry

##### Gene Structure and Function Group

1. Biosynthesis of ribosomes in animal cells with detailed analysis of rRNA methylation and pseudouridine. (Professor B. E. H. Maden)
2. Function of expression of U7 small nuclear RNA genes. (Dr. P. C. Turner)
3. Synthesis and application of 'caged' analogues of diadenosine tetraphosphate. (Dr. A. G. McLennan)
4. Identification of components of the protein translocational machinery in *E.coli*. (Dr. J. M. Pratt)

##### Molecular Oncology Group

5. Identification of putative gene(s) which upon transfection cause metastatic breast cancer in rodents. (Professor P. S. Rudland)
6. Isolation of cDNA clones for the mRNA of rat Fibroblast Growth Factor (FGF) receptor. (Dr. J. A. Smith)
7. Steroid hormone and anti-hormone regulation of specific gene expression in cultured breast epithelial cells. (Dr. C. D. Green)
8. Biochemistry and molecular biology of immune phagocytes in the role of these cells in inflammatory diseases including cancer. (Dr. S. W. Edwards)

##### Enzyme Regulation Group

9. Intracellular protein degradation in normal and pathological conditions. (Dr. R. J. Beynon)
10. The role of reversible phosphorylation in hormonal control processes. (Dr. M. J. Fisher)
11. Kinetic analysis of enzymes and the theory of pathway dynamics. (Dr. J. S. Easterby)
12. Kinetic analysis of enzymes involved in carbon fixation in chloroplasts. (Dr. R. Powls)

##### Isoprenoid Research Group

13. Hormonal control of arthropod development. (Professor H. H. Rees)
14. The regulatory role of sterol in plant cell division. (Dr. L. J. Goad)
15. Chemistry and biology of carotenoids. (Dr. G. Britton)
16. Biosynthesis and function of vitamin E in plants. (Dr. J. F. Pennock)

Contact: Dr. C. D. Green  
Tel: (051) 794 4365

#### Department of Environmental & Evolutionary Biology

1. Life history evolution in pests resistant to micropathogens. (Dr. M. E. Begon)
2. Population dynamics of damselflies. (Dr. D. J. Thompson)
3. Effects of pre-natal exposure to persistent pesticides on terrestrial mammals. (Dr. M. Stannistreet)

Further information may be obtained by writing to the named contacts at The University of Liverpool, P.O. Box 147, Liverpool L69 3BX, enclosing a curriculum vitae with the names and addresses of two referees, and indicating the research area(s) of interest.

4. Adaptive significance and physiological basis of phenotypic plasticity in grasses. (Dr. T. McNeilly)
5. Genetics of herbicide resistance in grass weed populations. (Dr. P. D. Putwain)
6. Assembly rules in arable plant communities. (Dr. A. M. Mortimer)
7. Ectocommensals and parasites as indicators of environmental change. (Dr. J. C. Chubb)
8. Interactions between periphytic algae and submerged macrophytes. (Dr. J. W. Eaton)
9. Analysis of multiple unknown organic contaminants in estuarine biota. (Dr. R. T. Leah)
10. The role of calcium in cellular control mechanisms. (Professor C. J. Duncan)
11. Cellular mechanisms of temperature and salinity adaptation. (Dr. A. R. Cossins)
12. Control of photosynthetic activity in cocoa. (Dr. K. Hardwick)
13. Role of the vacuole in salinity tolerance of marine algae. (Dr. J. C. Collins)
14. Appetite regulation in mammals. (Dr. D. M. Ensor)
15. Food value, feeding and temperature relationships in fish. (Dr. K. O'Hara)

Contact: Dr. A. M. Mortimer  
Tel: (051) 794 5127

#### Port Erin Marine Laboratory

16. Intraspecific competition in both natural and cultivated populations of seaweeds. (Professor T. A. Norton)
17. Aspects of the biology of juvenile scallops associated with cultivation. (Dr. A. R. Brand)
18. Genetic and ecological differentiation in *Actinia*. (Dr. J. P. Thorpe)
19. The ecology of the sublittoral fringe on rocky shores. (Professor T. A. Norton and Dr. J. M. Kain)
20. Micrograzing in the shallow subtidal algal undergrowth — how important? (Professor T. A. Norton)
21. Taxonomy and population genetics of *Patella* in relation to larval dispersal. (Dr. S. J. Hawkins and Dr. J. P. Thorpe)
22. Ecology and physiology of feeding in Antarctic bryozoa. (Dr. J. P. Thorpe and Dr. A. Clarke, (British Antarctic Survey))

Contact: Professor T. A. Norton  
Tel: (0624) 832027 Ext. 140

#### Department of Genetics & Microbiology

- ##### Animal Genetics
1. Molecular genetics of development in Mosquitoes. (Dr. P. Eggleston)

2. Cytogenetic studies and hypervariable DNA sequences in domestic animals/ effects of pollution. (Dr. J. J. B. Gill)
3. Molecular organization and activity of eukaryotic chromatin and chromosomes/ human cytogenetics. (Dr. R. S. Hill)

##### Fungal and Plant Physiology & Genetics

4. Control of gene expression in fungi. (Dr. M. X. Caddick)
5. Flavour synthesis in plants/fungal disease in plants. (Dr. H. A. Collin)
6. DNA repair and recombination in fungi/ genome organization in *Phytophthora*. (Dr. B. M. Faulkner)
7. Fungal disease in plants/biochemical properties of fungal protoplasts. (Dr. S. Issac)
8. Physiology and development in fungi including nutrition. (Professor D. H. Jennings)
9. Osmolyte response to environmental changes in fungi. (Dr. M. G. Jones)
10. Biochemistry and genetics of metal binding proteins and metal tolerance in plants and fungi. (Dr. D. A. Thurman)
11. Molecular genetics of metal binding proteins and metal tolerance in plants and fungi. (Dr. A. B. Tomsett)

##### Microbiology & Microbial Genetics

12. Molecular biology of thermophilic bacteria/fate of genetically-engineered-microorganisms (GEMs) in the environment. (Dr. C. Edwards)
13. Significance of antibodies to *Klebsiella* infections. (Dr. R. J. Jones and Dr. E. A. Roe)
14. Biology of *Actinomycetes* and biodegradation by bacteria/fate of GEMs in the environment. (Dr. A. J. McCarthy)
15. Biochemistry and enzyme activity of certain protozoa and bacteria/microbial decaffeination. (Dr. P. G. G. Miller)
16. Microbiology of waste water treatment and pollution. (Dr. H. W. Pearson)
17. Molecular genetics of antibiotic production in *Streptomyces* — toxic heavy resistance. (Professor D. A. Ritchie)
18. Bacterial pathogenicity and antigenic variation/fate of GEMs in the environment. (Dr. J. R. Saunders)
19. Molecular genetics of DNA repair in bacteria and fungi/toxic heavy metal resistance in bacteria. (Dr. P. Strike)
20. Biology, genetic manipulation and phenetic identification of *Streptomyces*. (Professor S. T. Williams)

Contact: Dr. R. S. Hill  
Tel: (051)-794 3628



# Sandoz Prize for Immunology

The prize will be worth US \$ 100 000 (US \$ 20 000 personal recognition/US \$ 80 000 support for research programme), and will be sponsored by SANDOZ LTD., Basle, Switzerland with the purpose of encouraging research in all areas of immunology with special emphasis on clinical immunology, including autoimmune diseases, cancer immunology, immunity to infectious diseases, transplantation immunology and discoveries in immunology leading to therapeutical applications.

Members of the Jury are G. Ada, J.-F. Bach, T. Honjo, P. Marrak, H.O. McDevitt, J.J. van Rood, R. Zinkernagel, and two representatives of Sandoz Ltd. The prize will be awarded on the occasion of an important Immunology Meeting in 1990.

Applications in English should comprise a summary of the research work of 3–5 pages, curriculum vitae, bibliography, experimental original papers separate from reviews and chapters, and reprints of not more than 3 key published papers in English or with extended summaries in English.

Individuals and research teams are invited to submit their applications not later than 30th June, 1989 to Sandoz Prize for Immunology, P.O. Box 182, 4013 Basel, Switzerland.



(W5985)N

## ASSISTANTSHIPS

### UNIVERSITY OF STRATHCLYDE DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY **RESEARCH ASSISTANT**

Applications are invited from graduates, preferably with PhD degrees in physiology and pharmacology or a related subject for a two-year industrially funded Research Assistantship to work on a project concerned with mechanisms of action of skeletal muscle relaxants used in anaesthetic practice. Experience in intracellular recording or ligand binding techniques would be an advantage. Salary: in range £8,675–£15,720 per annum.

Applications (Ref. R20/89) with full c.v. and the names and addresses of 3 referees should be sent to **Dr I G Marshall, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XQ**, who will be pleased to supply further information. Closing date for Applications:— 30 March 1989. (8803)P

### The University of Manchester **DEPARTMENT OF PSYCHIATRY** **RESEARCH ASSISTANTSHIP IN HUMAN PHARMACOLOGY**

A Research Assistantship is available in this Department for three years starting 1st October, 1989. The successful applicant will be expected to register for a PhD degree. He/she will work with a small research team on a project investigating autonomic pharmacology in healthy volunteers and patients suffering from alcoholism. The project involves both pharmacological and physiological measurements in human subjects in vivo, and biochemical assays on blood samples. Starting salary: £8,675 with superannuation benefits. Applicants should have a 1st or upper-2nd class honours degree in Pharmacology or Physiology. Applications, with a curriculum vitae and names of two referees, should be sent to

**Dr E. Szabadi, Department of Psychiatry, Stopford Building, The University, Manchester M13 9PT.** Closing date: 30th June, 1989. (8821)P

## STUDENTSHIPS continued

### THE UNIVERSITY OF WARWICK DEPARTMENT OF BIOLOGICAL SCIENCES **POSTGRADUATE RESEARCH STUDENTSHIPS**

Applications are invited as soon as possible for approximately 20 postgraduate studentships tenable in the fields of virology, microbiology, plant biochemistry, molecular biology and animal developmental biology from 1st October 1989 funded by the Research Councils (e.g. SERC and MRC) and other bodies. The following are examples:

- |                       |   |
|-----------------------|---|
| <b>CRC</b>            | 'The interaction of interferons with the immune response in resistance to malignant disease' (Dr. A. G. Morris)                             |
| <b>MAFF</b>           | 'Microbial growth and survival on food contact surfaces pertinent to food poisoning, spoilage and hygiene' (Dr. C. S. Dow)                  |
| <b>SERC/Earmarked</b> | 'Protein targeting in the Gram negative, plant pathogenic – <i>Erwinia carotovora</i> ' (Dr. G. P. C. Salmond)                              |
| <b>SERC/Earmarked</b> | 'Modified proteins and their role in controlling enzyme substrate specificity' (Prof. H. Dalton)  |
| <b>SERC/Earmarked</b> | 'Genetic manipulation of ricin for the development of recombinant immunotoxins' (Prof. J. M. Lord and Dr. L. M. Roberts) (two studentships) |

Applicants should have, or expect to have, a first or upper second class honours degree in an appropriate subject.

Further information can be obtained by consulting the Mini-UCCA handbook or from the **Postgraduate Admissions Tutor, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL**, (0203-523517) to whom applications, including a curriculum vitae and the names and addresses of two academic referees, should be sent indicating, where possible, the project of interest. (8813)F

## WORKSHOPS

### Carolina Workshops on GENERAL DNA TECHNOLOGY

**Sequencing & Mutagenesis**  
May 19–June 4, 1989

A general DNA techniques workshop including shotgun cloning into M13 vectors, dideoxy sequencing of random subclones and computer generation of sequence overlaps. Students may bring their own DNA for sequencing. High efficiency, oligonucleotide directed mutagenesis methods with phagemid vectors will be used for saturation mutagenesis of protein domains. Methods will be applied to a structure-function analysis of the *E. coli* maltose binding protein. Tuition is \$1600.

**Instructors:**

Alan Bankier, MRC, Cambridge,  
England

Richard Ogden, Agouron  
Institute, San Diego

Nelson Rhodes II, UNC-CH



The Program in  
Molecular Biology  
& Biotechnology

**Cloning cDNA into  
Expression Vectors**  
July 14–30, 1989

A general DNA techniques workshop in which synthesis of cDNA can cloning into lambda gt-11 are highlighted. Students may bring their own tissue or be provided with tissue from which to isolate mRNA. Students may bring antibody to screen for expression of the desired cDNA. Additional techniques include mRNA isolation, Northern transfer, Northern hybridization, and detection of proteins expressed in *E. coli*. Tuition is \$1600.

**Instructors:**

David Joseph, UNC-CH

Jerry Ware, Scripps Clinic,  
La Jolla

For further information or to apply, contact:

Dr. Susan J. Kelly, Workshop  
Coordinator

University of North Carolina at  
Chapel Hill

Program in Molecular Biology  
& Biotechnology

402 Swing Building CB 7100N  
Chapel Hill, North Carolina  
27599-7100N

CAROLINA WORKSHOPS are a series of intensive hands-on laboratory courses designed to teach cutting edge methods in DNA technology. The techniques include basic manipulations such as isolation of insert and vector DNA, ligations, electrophoretic analysis and elution from gels, blotting techniques, preparation and labeling of probes, and identification of cloned DNA. Each course provides experience in the basic techniques which serve as a solid, basic background in DNA technology. Each individual course has an identifiable theme and emphasizes in addition to the general techniques, specialized techniques such as DNA sequencing, in vitro mutagenesis, cDNA synthesis, and/or expression vectors. Students are given the opportunity to learn the techniques while also using their own starting materials or may choose to be provided with starting materials.

Many applicants to the Carolina Workshops already hold the M.D. or Ph.D. degree. Our courses are very heavy on hands-on contact with the protocols, light on lectures concerning the basics of molecular biology. Hence, a reasonable familiarity with the concepts behind DNA techniques such as those found in good texts (*e.g. Recombinant DNA: A Short Course* by Watson, Tooze, and Kurtz) is necessary to get a good yield from these courses. Experience with running these courses for seven years now indicates that both students with and students without any prior hands-on experience with molecular techniques find these courses useful. Experienced students benefit from in depth interaction with the instructors; novices learn the realities behind the innocent looking protocols. The results obtained in the Carolina Workshops can be considerable. In a recent Workshop one researcher generated 21 kb of sequence data in the Sequencing Workshop; another created 7 cDNA libraries in the cDNA Cloning Workshop.

To apply, write a brief letter describing your research interests and their relevance to the Workshop for which you apply. Please indicate your complete mailing address and telephone number. Applications received by April 7 will receive full consideration. (NW3507)V

## GRANTS & SCHOLARSHIPS

### UNIVERSITY OF EDINBURGH FACULTY OF VETERINARY MEDICINE JAMES TINDAL POSTGRADUATE SCHOLARSHIP

The Faculty will be awarding this Scholarship from October 1989 to a candidate for a research degree in the Faculty. The scholarship will comprise payment of tuition fees as well as a maintenance allowance at the University's standard rate. The award is for one year in the first instance but may be renewed up to a maximum of three years subject to satisfactory progress. Further details, application forms, and details of the Faculty's current research activities may be obtained from the **Faculty Officer, University of Edinburgh, Faculty of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh EH9 1QH.**

Please quote reference: N.5651.

Application forms must be returned by 1 May 1989.

(8791)H

## ANNOUNCEMENTS

### FEBS '89 — 19th MEETING OF THE FEDERATION OF BIOCHEMICAL SOCIETIES — ROME, JULY 2/7, 1989

The Organizing Committee informs that the Secretariat numbers (Tel. 0039/6/3960341, Fax 0039/6/3964377) have changed to **TEL. 0039/6/3221806, FAX 0039/6/3222006.**

(W6003)G

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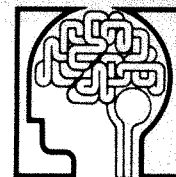
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# FIDIA RESEARCH FOUNDATION

is honored to participate in the celebration of the  
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Bicentennial Lecture on "Artificial Intelligence" and the Bicentennial Symposium  
"Neuroscience in the Twenty-First Century: New Perspectives and Horizons."



Monday, April 17, 1989 at 3:30pm

Georgetown University, Gaston Hall, Washington, DC

Bicentennial Lecture on

## ARTIFICIAL INTELLIGENCE

Gerald M. Edelman, Nobel Laureate,

Vincent Astor Professor at The Rockefeller University, will speak on:

### "CONCEPTS OF DARWINIAN SELECTION AND BRAIN FUNCTION"

Gerald M. Edelman will be introduced by Erminio Costa, Fidia-Georgetown Institute for the Neurosciences

The Lecture will be followed by a Round Table with the participation of:

**W. Maxwell Cowan**, Moderator  
Howard Hughes Medical Institute, Bethesda, MD

**Richard Michod**, Department of Ecology and  
Evolutionary Biology  
University of Arizona, Tucson, AZ

**Vernon B. Mountcastle**, Department of Neuroscience  
The Johns Hopkins University, Baltimore, MD

**Tomaso A. Poggio**, Department of Psychology  
Massachusetts Institute of Technology, Cambridge, MA

**Pasko Rakic**, Section of Neuroanatomy  
Yale University, New Haven, CT

April 22-24, 1989

Georgetown University Conference Center, Washington, DC

Bicentennial Symposium

## "NEUROSCIENCE IN THE TWENTY-FIRST CENTURY: NEW PERSPECTIVES AND HORIZONS"

Saturday, April 22, Morning

### LECTURA MAGISTRALIS

**Rita Levi Montalcini**, Rome, Italy  
NERVE GROWTH FACTOR AND  
NEURONAL PLASTICITY

**MODERATOR: Lawrence Kromer**,  
Georgetown University

**SPEAKERS: Lloyd Green**, New York, NY  
**Italo Mocchi**, Washington, DC  
**Eugene Johnson**, St. Louis, MO  
**Lawrence Kromer**, Washington, DC

Sunday, April 23, Morning

### LECTURA MAGISTRALIS

**Marshall Nirenberg**, Bethesda, MD  
ASPECTS OF REGULATION OF  
GENE EXPRESSION IN NEURONAL CELLS

**MODERATOR: Erminio Costa**,  
Georgetown University

**SPEAKERS: Shigetada Nakanishi**, Kyoto, Japan  
**Robert H. Costa**, Chicago, IL  
**James I. Morgan**, Nutley, NJ  
**Anna Maria Szekely**, Washington, DC

Monday, April 24, Morning

### LECTURA MAGISTRALIS

**Roger C. Guillemin**, San Diego, CA  
THE BRAIN PEPTIDES CONTROLLING PITUITARY  
FUNCTION AND MORE

**MODERATOR: Alessandro Guidotti**,  
Georgetown University

**SPEAKERS: Michael Comb**, Boston, MA  
**Dan Lanhammar**, Uppsala, Sweden  
**Claes Wahlestedt**, Washington, DC  
**Alessandro Guidotti**, Washington, DC  
**Solomon H. Snyder**, Baltimore, MD

Saturday, April 22, Afternoon

### LECTURA MAGISTRALIS

**Julius Axelrod**, Bethesda, MD  
CATECHOLAMINE NEUROTRANSMITTERS

**MODERATOR: Jarda T. Wroblewski**,  
Georgetown University

**SPEAKERS: Irwin J. Kopin**, Bethesda, MD  
**Marc G. Caron**, Durham, NC  
**Donald J. Reis**, New York, NY  
**Martin Rodbell**, Research Triangle Park, NC

Sunday, April 23, Afternoon

### LECTURA MAGISTRALIS

**D. Carleton Gajdusek**, Bethesda, MD  
REPLICATING AMYLOIDOSES: UNCONVENTIONAL  
SLOW VIRUSES AND DEMENTIA

**MODERATOR: Norman P. Salzman**,  
Georgetown University

**SPEAKERS: Anthony S. Fauci**, Bethesda, MD  
**John F. Griffith**, Washington, DC  
**Michael Oldstone**, La Jolla, CA

Monday, April 24, Afternoon

### ROUND TABLE

**Torsten N. Wiesel**, New York, NY  
FUNCTIONAL ORGANIZATION  
OF THE STRIATE CORTEX

**MODERATOR: Stefano Vicini**,  
Georgetown University

**SPEAKERS: Denis A. Baylor**, Stanford, CA  
**Elio Raviola**, Boston, MA  
**Lamberto Maffei**, Pisa, Italy  
**Carla J. Shatz**, Stanford, CA  
**Tomaso Poggio**, Boston, MA

The symposium will be held from 9:00am to 12:45pm and from 2:00pm to 5:30pm at Georgetown University Conference Center. The registration fee is \$150.00 (\$75.00 for students and postdoctoral fellows) and includes lunches. Registration deadline: **March 31, 1989**. For further information contact:

(NW3491)M

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## Molecular Communication in Higher Plants 18-21 September 1989 at EMBL, Heidelberg, F. R. Germany

### Invited speakers include:

D. Baulcombe (GB-Norwich), M. Bennett (GB-Kew), M. Bevan (GB-Cambridge), T. Bisseling (NL-Wageningen), U. Bonas (D-Berlin), C. Bowler (B-Gent), F. de Bruijn (D-Köln), M. Caboche (F-Versailles), N.-H. Chua (USA-New York), A. Clarke (Australia-Melbourne), E. Coen (GB-Norwich), G. Coruzzi (USA-New York), J. Dangl (D-Köln), E. Dennis (Australia-Canberra), A. Gatenby (USA-Wilmington), W. Gerlach (Australia-Canberra), A. Gierl (D-Köln), R. Goldberg (USA-Los Angeles), W. Gruissem (USA-Berkeley), R. Hedrich (D-Göttingen), T. Hohn (CH-Basel), R. Horsch (USA-St. Louis), D. Jofuku (B-Gent), J. Jones (GB-Norwich), J. Leemans (B-Gent), P. Meyer (D-Köln), D. Mohnen (USA-Athens), T. Nelson (USA-New Haven), K. Palme (D-Köln), I. Potrykus (CH-Zürich), P. Quail (USA-Albany), E. Schäfer (D-Freiburg), A. Sievers (D-Bonn), C. Somerville (USA-East Lansing), A. Spena (D-Köln), D. van der Straeten (B-Gent), A. Trewavas (GB-Edinburgh), S. C. de Vries (NL-Wageningen), E. Weiler (D-Bochum), P. Weisbeek (NL-Utrecht), U. Wienand (D-Köln), L. Willmitzer (D-Berlin).

### There will be nine plenary sessions which will cover:

Cell-cell interactions; Differential gene expression/mutational analysis of plant function; Inter and intracellular signalling and the regulation of plant growth; Plant and environment; Plant biotechnology; Plant and microbes; Plants and viruses; Protein traffic and assembly into higher order structures; Structure, modification and expression of the nuclear genome (including novel techniques for gene isolation). The two poster sessions will allow participants to present their work.

### Registration:

The Symposium will be at the European Molecular Biology Laboratory, Heidelberg, with registration on Sunday, 17 September 1989. The registration fee, which includes daily transport to and from the EMBL, lunches, and the Symposium reception, is DM 180, for graduate students DM 90, and for participants from industry DM 360. Participants will be accommodated in the EMBL guest house and hotels in Heidelberg. The registration fee does NOT cover the cost of accommodation.

### Application:

The deadline for applicants is 16 June 1989. Applications should include a curriculum vitae and a brief description of research interests. The organizing committee will notify those who have been accepted as soon as possible after the deadline. The total number of participants will be limited to 250. Applications should be addressed to **Dr. J. Tooze, EMBO, Postfach 1022.40, D-6900 Heidelberg, F. R. Germany**. Applicants wishing to present a poster should send a 1-page abstract together with the registration fee and reply sheet after they have been accepted for participation.

### Organizing committee:

C. Leaver (Edinburgh) -Chairman-, K. Marcker (Aarhus), M. van Montagu (Ghent), I. Potrykus (Zürich), H. Saedler (Köln), F. Salamini (Köln), J. Tooze (EMBO, Heidelberg). (W5907)M

## CONFERENCES

## An International Conference NEW APPROACHES TO THE TREATMENT OF ALLERGIC DISEASE

19th/20th June 1989

Royal College of Physicians, London

This conference will provide an authoritative overview of the current status of research into the mechanisms of allergic diseases such as rhinitis, urticaria and asthma. It will also provide an up-to-date account of the current thinking on the treatment of allergic diseases and the prospects for future drug development.

### Chairmen and Speakers:

**Professor S. T. Holgate**  
Southampton General  
Hospital, UK.

**Dr. R. J. Davies**  
St. Bartholomew's Hospital, UK.

**Dr. J. Morley**  
Sandoz AG, Switzerland.

**Dr. L. G. Garland**  
The Wellcome Research  
Laboratories, UK.

**Dr. R. McMillan**  
ICI Pharmaceuticals, UK.

**Dr. B. R. Allen**  
Queen's Medical Centre  
Nottingham, UK.

**Dr. N. Barnes**  
The London Chest Hospital, UK

**Dr. L. Michel**  
Hôpital Henri Mondor, Créteil,  
France.

For further information and registration details,  
please contact:

**Renata Duke, IBC Technical Services Ltd., Bath House**  
(3rd Floor), 56 Holborn Viaduct, London EC1A 2EX  
Tel: 01-236 4080. Fax: 01-489 0849  
Telex: 888870 IBC G.

**Professor A. B. Kay**  
National Heart and Lung  
Institute, London, UK.

**Dr. D. K. Rainey**  
Fisons Pharmaceuticals, UK.

**Dr. C. De Vos**  
UCB Pharmaceutical Sector,  
Belgium.

**Dr. D. Proud**  
The Johns Hopkins University  
School of Medicine, USA.

**Dr. D. R. Stanworth**  
University of Birmingham, UK.

**Dr. H. Smith**  
Beecham Pharmaceuticals, UK.

**Dr. D. R. Buckle**  
Beecham Pharmaceuticals, UK.

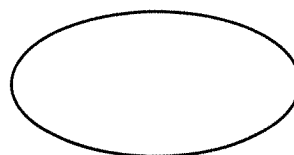


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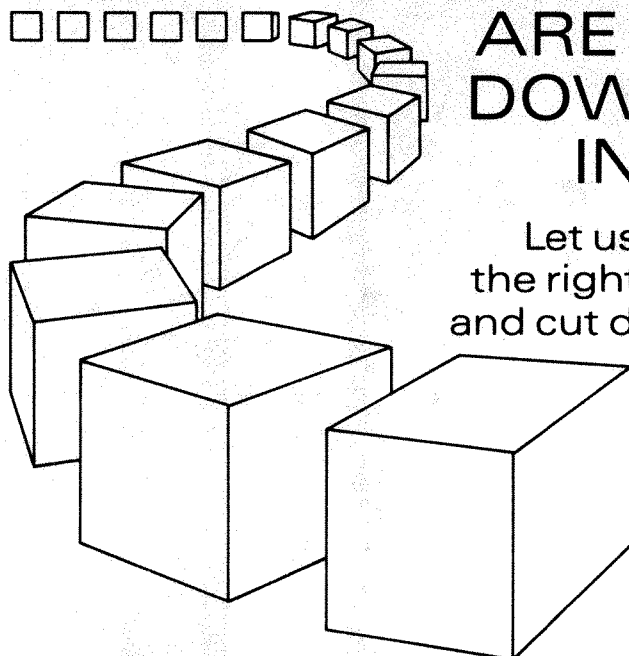
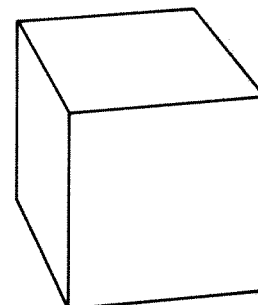
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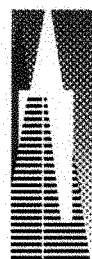
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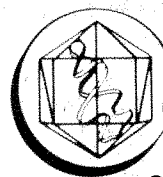
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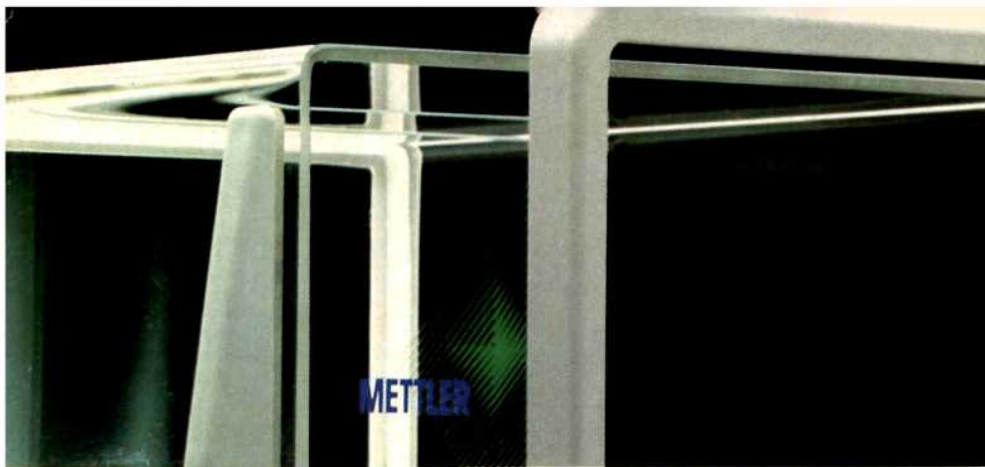
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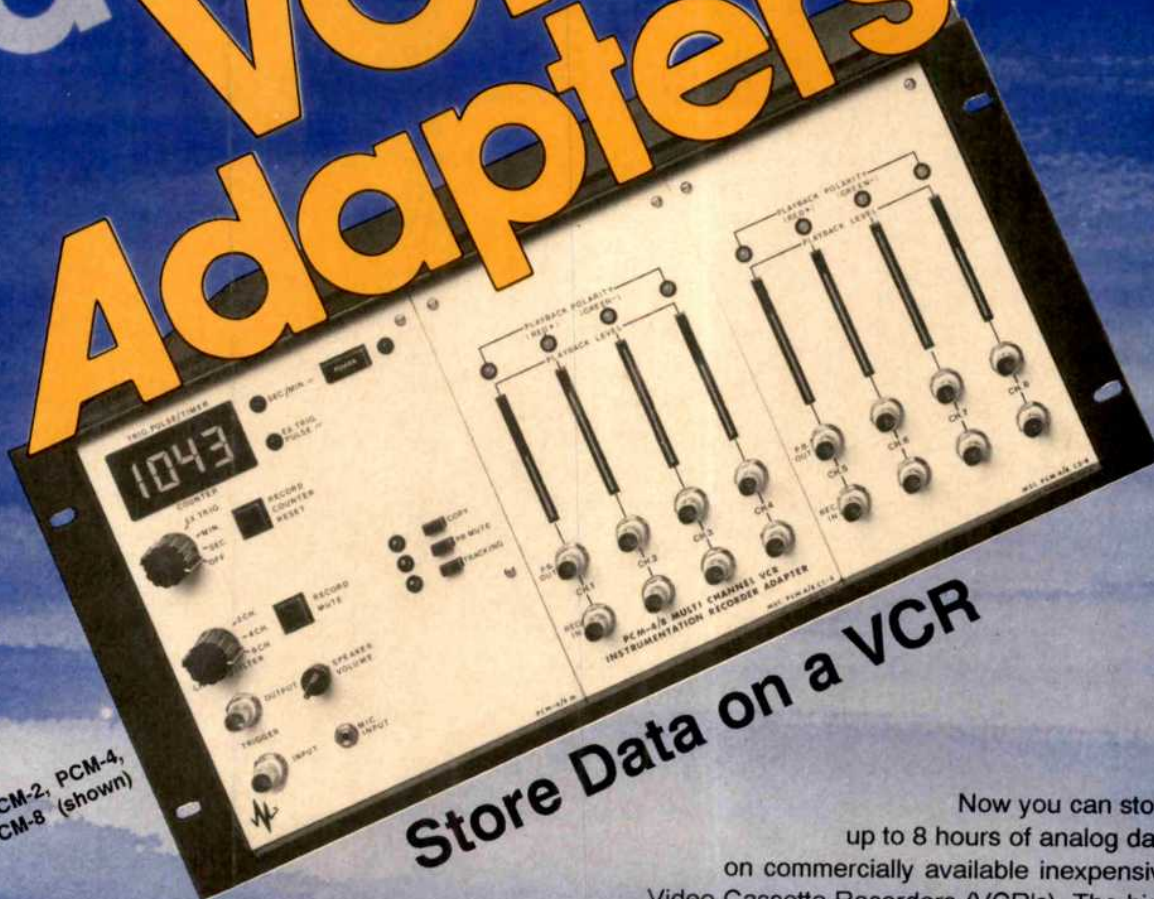
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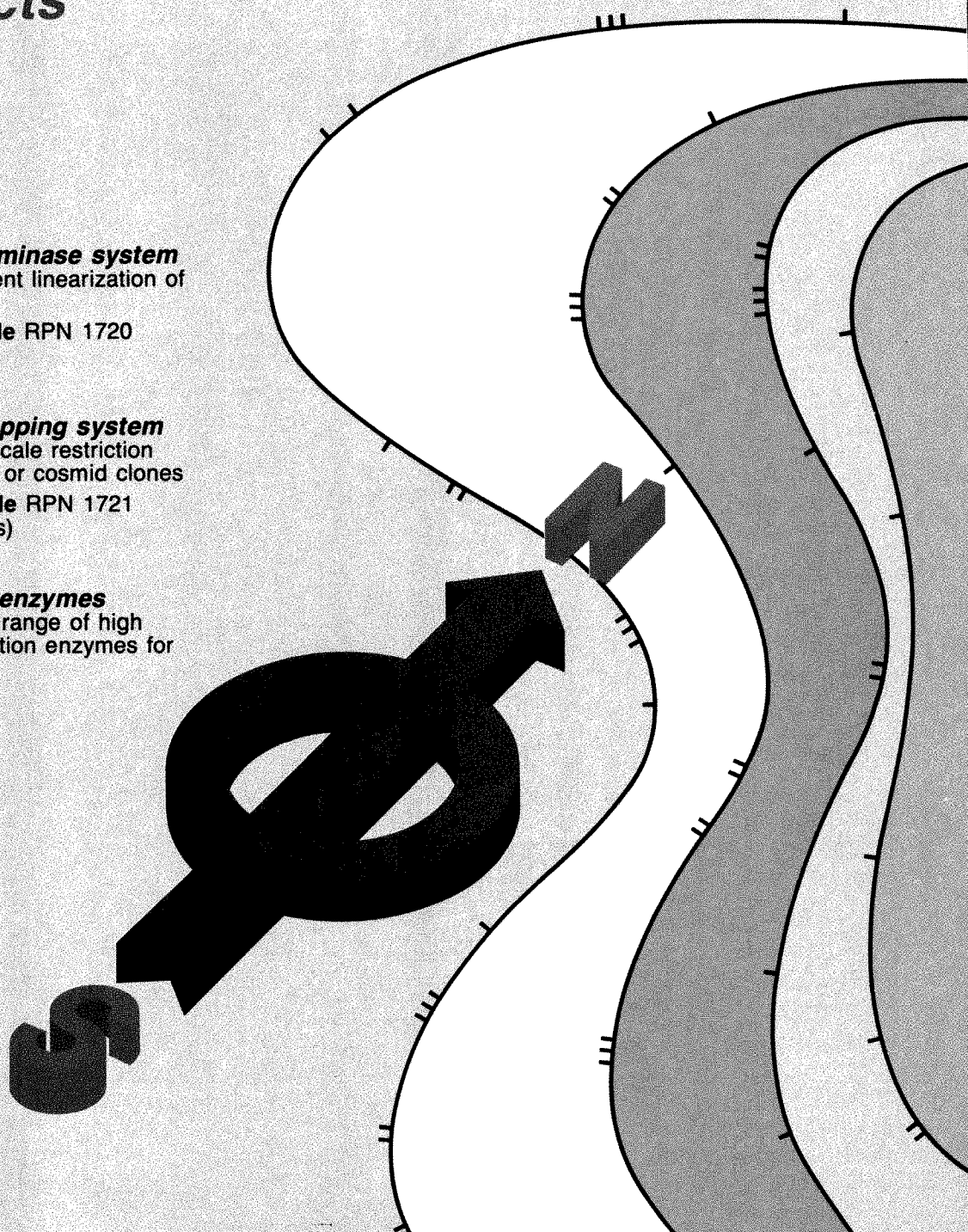
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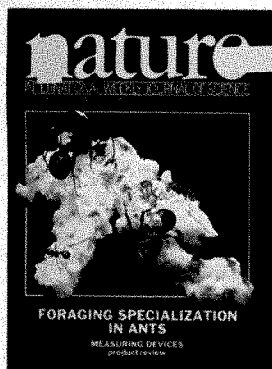
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# nature

30 March 1989  
Vol. 338 Issue no. 6214

In the leaf cutter ant *Acromyrmex versicolor*, queens aggregate and co-operate in the absence of kinship contrary to expectations based on kin selection theory (page 420 and News and Views). The cover shows several forager ants of this species, together with the larger queens.

## THIS WEEK

### That fusion story . . .

Comment on page 361; the background, page 364.

### Greater volume

Stephen Jay Gould on "one of the greatest causes for celebration in the history of publishing", page 385. Opinion, page 362.

### Sea change

Unexpected changes in a range of properties of water in the Black Sea lead to the suggestion that man's activities are seriously altering the ecological balance of this marine basin. Page 411.

### Disease and life style

Epidemiology implicates both hygiene and diet in diseases of Western civilization, including cancer of the colon. Environmental factors in cancer of the breast have been more difficult to identify. Commentary, page 371 and Review Article, page 389.

### Temperature rises . . .

Simply exposing a YBCuO superconductor to nitrogen can increase its transition temperature — or can it? See page 383.

### Glycine excitement

On page 425, how glycine exerts its modulatory effect on the NMDA-activated ion channel, one of the receptors for the neurotransmitter glutamate. And on page 422, the effect of the modulation demonstrated in nervous tissue. The significance of these results is discussed on page 377.

### On guard

The effect of changes in calcium concentration on potassium currents across the plasmamembranes of plant guard cell protoplasts supports the suggested role for calcium in the control of stomatal opening. Page 427.

## Meteoritic impact

The presence of 'diaplectic' glass produced without fusion is generally considered to be evidence of impact-induced shock, such as the impact of meteorites on the Earth and the Moon. But high pressure and low temperatures can produce a similar effect in the laboratory without compression due to impact. Page 413.

## Ubiquitin: new role

A new function for the protein ubiquitin is suggested by the finding that the 'tails' that extend the carboxy-termini of the products of three of the four ubiquitin genes code for essential ribosomal proteins. Pages 394 and 379.

## A 'ringing' Sun

Tree-ring and carbon-14 dating provide a chronology going back 9,600 years and raise the possibility of a 420-year fundamental period for variations of climate and solar activity, page 405.

## Paternity insurance

Mate guarding by male Idaho ground squirrels enhances a male's probability of paternity



which would otherwise be endangered by secondary matings and sperm competition. Page 418.

## Guide to Authors

Facing page 384.

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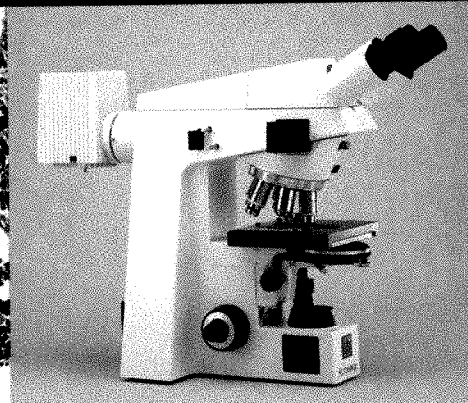
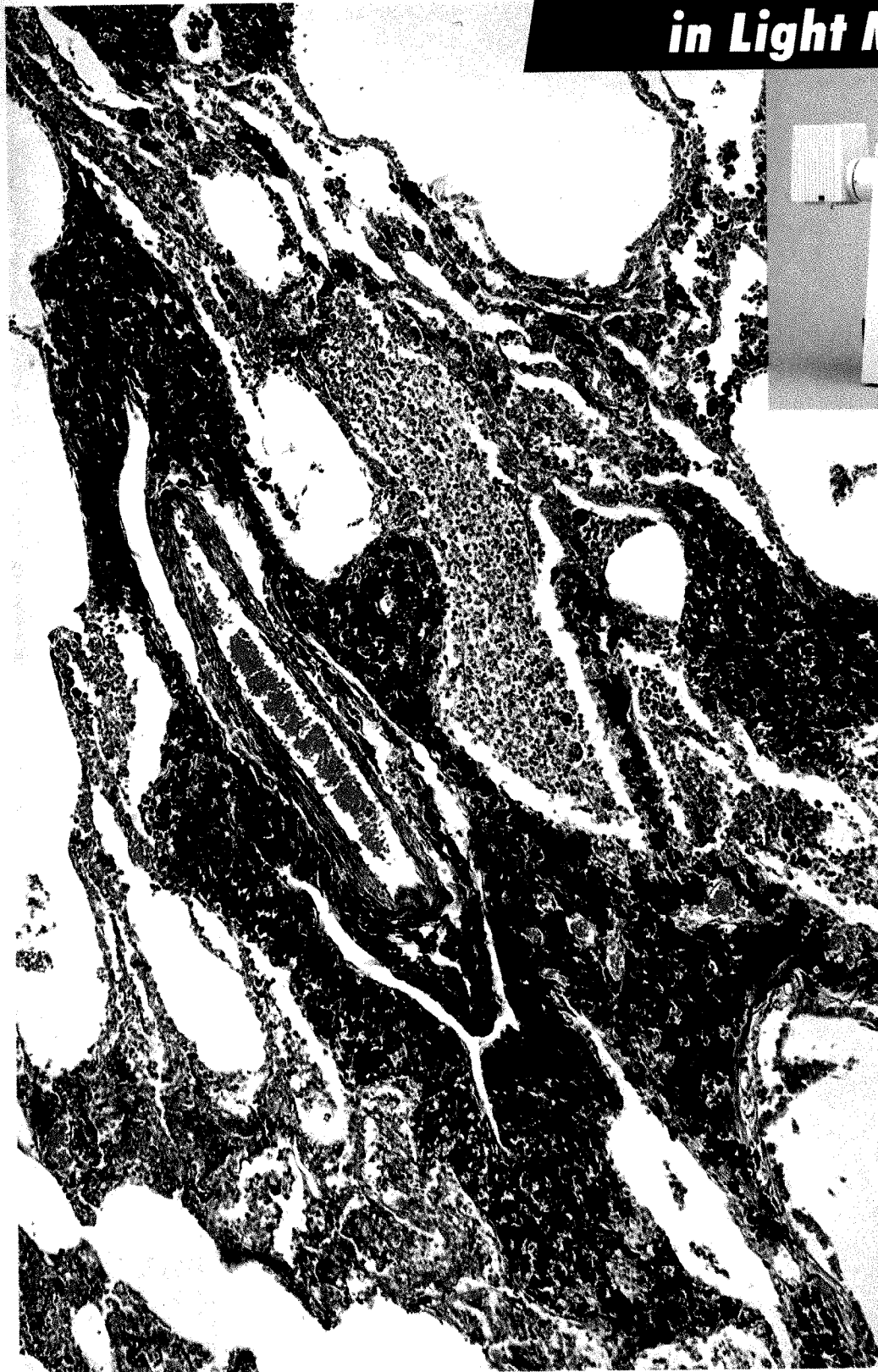
## BOOK REVIEWS

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7321 Lauterstein, W. Germany.

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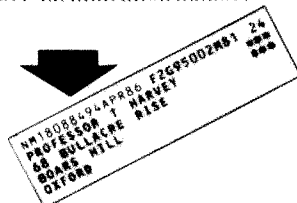
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<sup>1</sup>Kretz, P.L. and Short, J.M. (1989) *Strategies* Vol. 2 No. 2

NAME	CHARACTERISTICS	CAT#
P2PLK17	RecA <sup>+</sup> , mcrA <sup>-</sup> , mcrB <sup>-</sup> , Spi Selection, lac <sup>-</sup> , hsdR <sup>-</sup> M <sup>+</sup>	200
PLK-F <sup>-</sup>	RecA <sup>+</sup> , mcrA <sup>-</sup> , mcrB <sup>-</sup> , hsdR <sup>-</sup> M <sup>+</sup> , F' (lacZ Δ15, lacI <sup>q</sup> )	200
PLK17	RecA <sup>+</sup> , mcrA <sup>-</sup> , mcrB <sup>-</sup> , lac <sup>-</sup> , hsdR <sup>-</sup> M <sup>+</sup>	200
CPLK	RecA <sup>+</sup> , lac <sup>-</sup> ( <i>E. coli</i> C)	200
P2CPLK	RecA <sup>+</sup> , lac <sup>-</sup> , Spi selection, ( <i>E. coli</i> C)	200



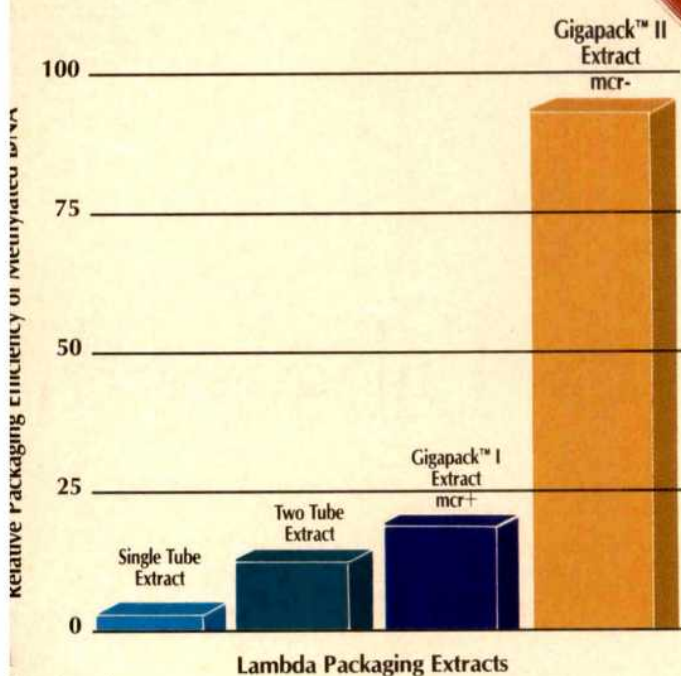


Figure Legend:

Highly methylated eukaryotic DNA was packaged with Gigapack II extract and three other commonly used lambda packaging extracts. The packaged DNA was then plated with the methylated cytosine restriction minus (mcr-) *E. coli* strain PLK17. The relative packaging efficiencies are shown.

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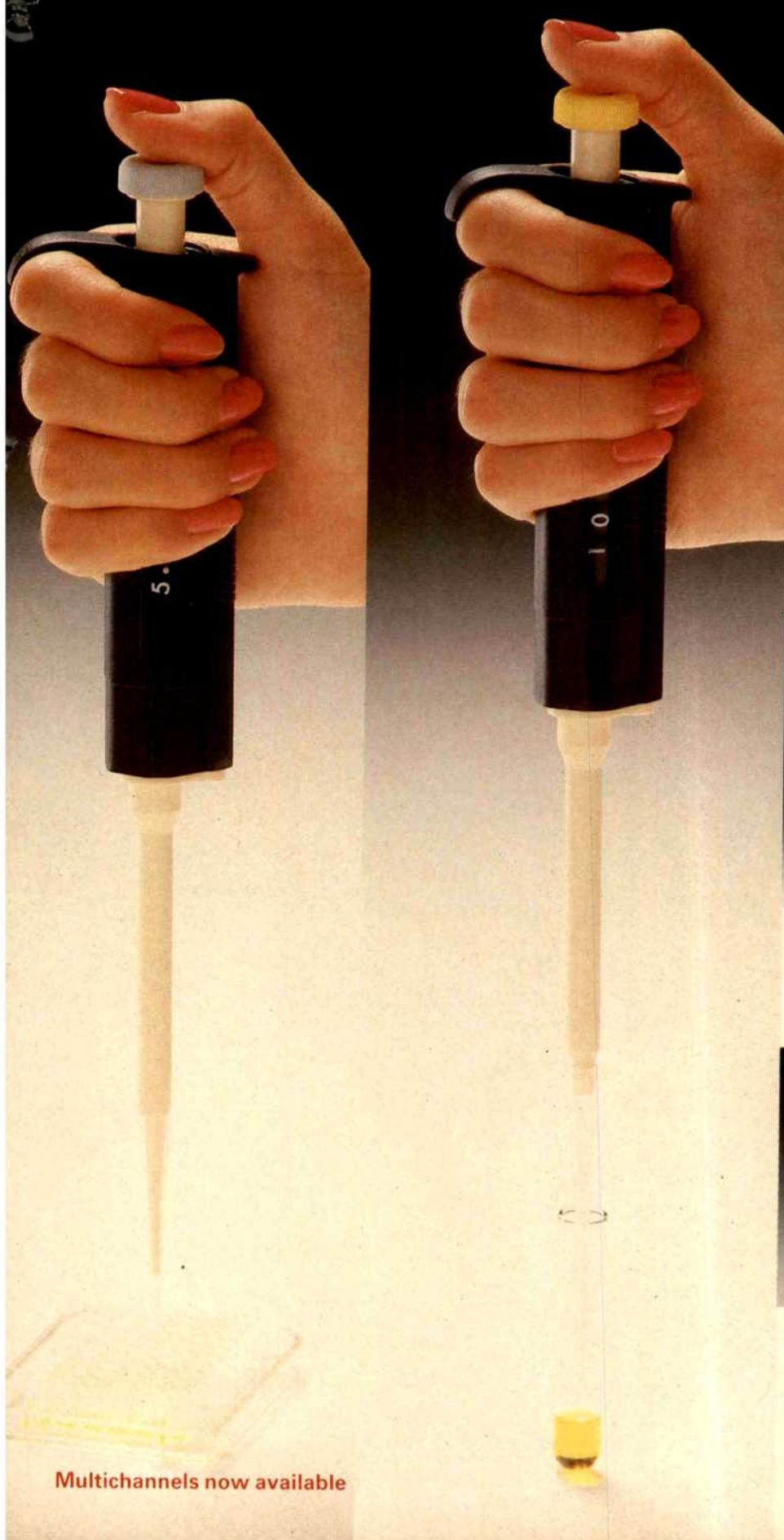
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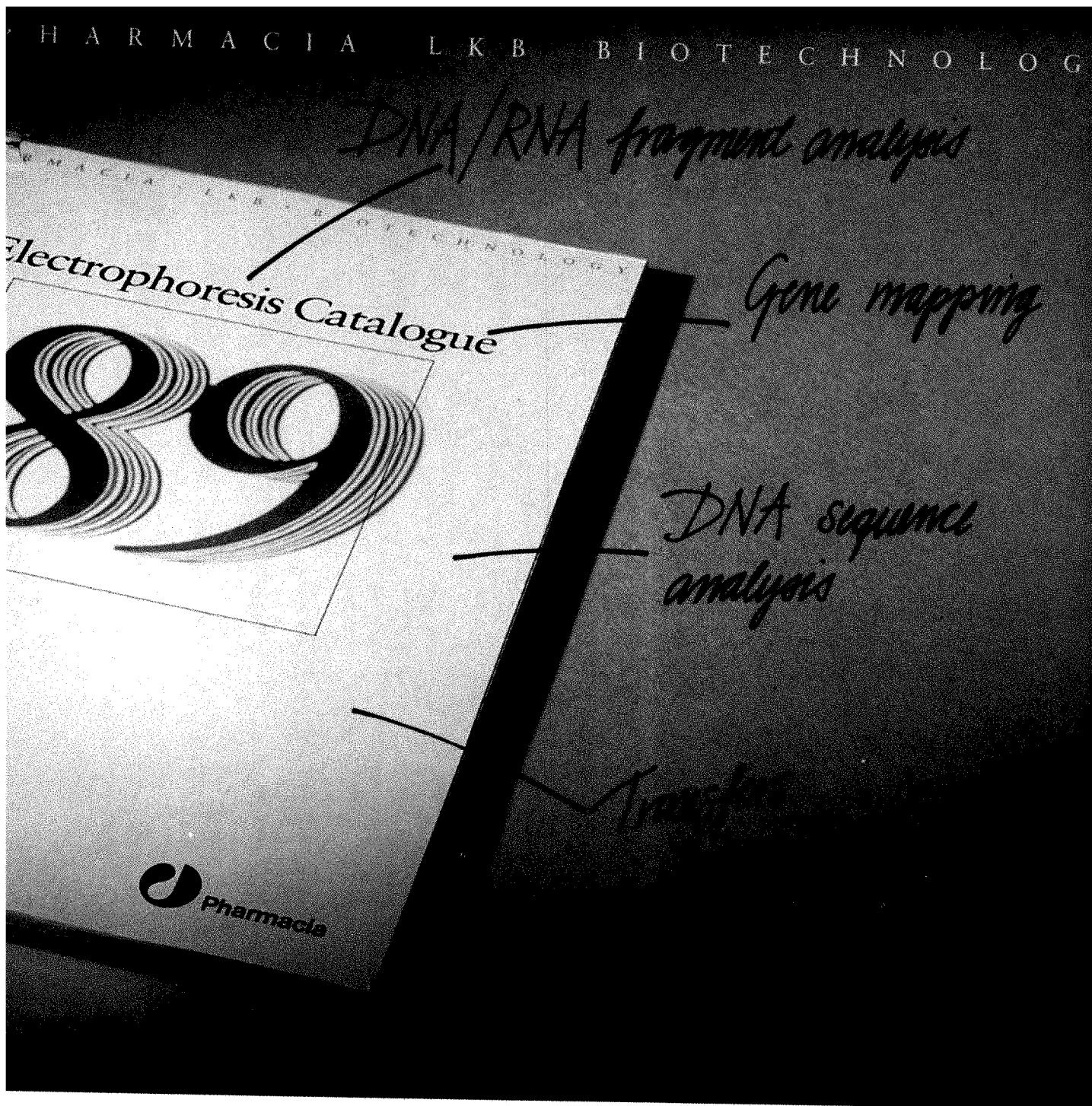
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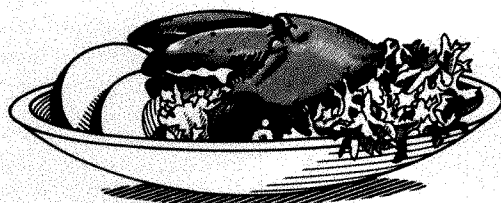
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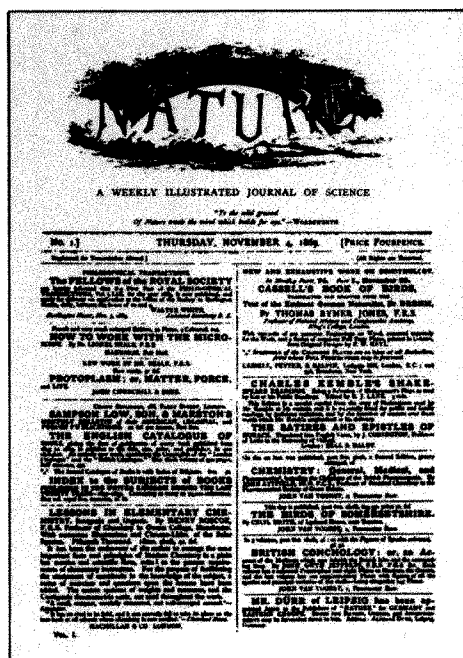
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## Academy elections in a muddle

The Soviet Academy of Sciences has been made a laughing-stock by the muddle over its constituency election for the Supreme Soviet. But the difficulty should have been anticipated.

THE continuing pantomime (see page 364) of the failure of the Soviet Academy of Sciences to elect a score of people as its representatives in the new Chamber of the People's Deputies is neither a surprise nor a criticism of the academy. On the contrary, it may more properly be regarded as a sign of grace. The academy's failure even to nominate enough people to fill the 25 places it had been offered in the new chamber (in the end, there were only 23 nominees, so that five places were relinquished) is a measure of the diversity of opinion within the academy, and of its members' willingness to behave as they believe. Is that not a virtue, rather than the opposite?

Even so, the conduct of the election, and its outcome, will not enhance the academy's reputation within the Soviet Union or elsewhere. Nor will it do much for the reputation of the Soviet electoral system, hastily devised last year as part of the cutting edge of *perestroika*. There are too many wheels within wheels for anybody's comfort. One difficulty is that there are too many kinds of constituencies. Some are geographical, some geographical constituencies include groups of others, while other constituencies are professional (such as the academy) or defined by other special interests (such as the company of Soviet philatelists). Another difficulty is that the rules by which professional and other societies have approached the process of election have been vague, to say the least.

Within the academy, the first step (in January) was a kind of popularity contest in which individual institutes put forward names for nomination. Sakharov and Sagdeev (in that order) came out on top, but then failed to win half the votes that might have been cast at the special conference a few weeks later. Now, 15 out of the 23 nominees still in the race have fallen for the same reason, no doubt victims of the advertised determination of those who wished to vote for Sakharov and Sagdeev to vote against all other candidates. Why, despite all the secret ballots, should candidates overwhelmingly popular at one stage of the election fail to be elected at the next? The immediate explanation is that the electorate changed (and narrowed) between the beginning of January and the end. And the explanation of that is that the academy, while seeking to give democratic processes an airing, did not dare go far down the road of one man, one vote.

Yet the Chamber of Deputies being elected is an important part of a new and more democratic system. Although it will meet only infrequently, it will have the crucial task of choosing the new Supreme Soviet, intended to become a full-time legislature under Mr Mikhail Gorbachev himself, who will be its president. It will have influence, even power. All the more reason why it should seem legitimate. Perhaps its first task should be to revise the law under which it is being elected, in the process abolishing the professional constituencies and the underlying principle of "most men, one vote: some men, two votes".

For the Soviet academy, the lessons to be drawn from this affair go even deeper. In the Soviet Union now, elections for institute directors, laboratory heads and others are all the rage. But on a matter such as the election of people to the Chamber of Deputies, which has nothing to do with the management of the academy's own over-complicated business, it is absurd that the academy should have canvassed the wishes of those working in its institutes and then have allowed them to be denied. If laboratory workers want Sakharov and Sagdeev to represent them, why should they not have their way? □

## Cold (con)fusion

Reports that an account of cold nuclear fusion is soon to appear in this journal are premature.

INCREASINGLY alert coverage of science by newspapers, taken as a sign of increasing public interest, is something that readers of this journal should applaud. But when scientists find themselves reading about their colleagues' discoveries in newspaper columns before anything has been submitted, let alone accepted or published, in a research journal, there is cause to be worried. No one was more surprised than the editors of *Nature* to learn, on reading last Friday's *Wall Street Journal* that two papers on room-temperature nuclear fusion (see page 364) would probably appear simultaneously in this journal, perhaps in May.

That editors of scientific journals are annoyed by this kind of event is due neither to sour grapes nor to a cabalistic devotion to secrecy. The procedure of peer-

review, slow and irksome as it sometimes can be, has evolved to protect not only journals but scientists and science itself from a barrage of unsubstantiated claims which later have to be withdrawn. The more outlandish the claim, the less likely authors seem willing to submit to careful scrutiny by knowledgeable people working in related areas. There are understandable reasons for this reticence. It is the rare piece of research indeed that both flies in the face of accepted wisdom and is so compellingly correct that its significance is instantly recognized. Authors with unique or bizarre approaches to problems may feel that they have no true peers who can evaluate their work, and feel more conventional reviewer's mouths are already shaping the word "no" before they give the paper much attention. But it must also be said that most unbelievable claims turn out to be just that, and reviewers can be forgiven for perceiving this quickly.

But unsubstantiated claims often make interesting news, which journalists have a duty to report even if they do so sceptically. Unlike other 'news' stories that appear in daily newspapers, 'science news' stories have no clear date when they become news worth printing. Is a scientific discovery news from the day it is made, the day it is submitted to a journal, the day the journal accepts it, or the day it is published? Reasonable people will differ on which is the correct answer.

Even with the best of luck and planning, this journal cannot take a scientific paper from receipt to publication in less than about a month. Last week's issue contains two examples, concerning the new pulsar in supernova 1987A, in which the researchers concerned were able to keep quiet for the required few weeks. But there are those who feel compelled, either for money or fame, to ballyhoo discoveries before anyone can reasonably judge their merits. Superconductors, AIDS treatments and new energy sources bring behind them patent lawyers and venture capitalists, to whom breaking the press embargo of an academic journal probably seems a negligible misdemeanour.

But patents that turn out to be worthless and investments that disappear may demonstrate the value of cautious peer-review to those who now think of it as a fusty institution much loved by pedants. The sceptical tone adopted in many newspaper accounts of cold fusion, and the emphasis given to the fact that no paper has yet been accepted for publication in any scientific journal, indicates that journalists (who learn nearly as fast as they write) appreciate the pitfalls. Many learned a lesson two years ago, when reports of superconductivity at increasingly incredible temperatures were appearing daily. It did not take long for science writers to realize something was amiss, and after seeking professional advice they began asking for specific evidence of superconductivity, such as magnetic-flux expulsion, in addition to claims of sudden drops in resistance. An obvious need existing, peer-review was spontaneously re-invented. □

## Words by machine

Computerizing the *OED* should help its publishers to remedy the dictionary's most obvious defects.

STEPHEN J. Gould is right to welcome the new edition of the *Oxford English Dictionary* as a landmark in the intellectual life of the English-speaking world (see page 385). It may be even more than that. That the dictionary of a language should be constructed on historical principles, recording all the usages of all words without the disparagement of any, was in itself a radical notion even further out of tune with the mood of Victorian England when the dictionary was begun than with the still-prescriptive inclinations of our times. The result, as Oxford University Press is eager to proclaim, is not so much a dictionary as a living record of the changing English language, or at least of that part of it used by the British. Will the appearance of the new edition of the dictionary spark the imitation by other-tongued linguistic chauvinists that could further augment its value by providing comparative records of how languages evolve?

For the British (and for the dictionary's publishers), several questions arise. The dictionary now published as ink on paper is not the real thing, but a first approximation to it. Words and meanings are accumulating all the time. The publishers plan to put out another version in a few years on compact disks readable by laser. No doubt successive versions will be numbered much as software manufacturers sell new versions of their products, in a decimal notation. Because the dictionary is built on historical principles, that should not be as confusing as it seems: new versions will include their predecessors. But, now that the Oxford University Press has survived its first serious brush with computer technology, there is also a case for making the dictionary accessible on-line. There is also now an opportunity to use the new technology for capturing examples of the use of new words, or of old words in new meanings, whose compilation has been the back-breaking and sometimes disheartening part of the labours of the past century. It is not merely that the publishers will thereby be saved time and money, but that they will then be less likely to neglect the maintenance of a important scholarly resource than they were in the 1930s, when work on the dictionary was halted for a quarter of a century.

That is also how best to make progress towards remedying even the new edition's most obvious deficiencies, of which there are two. First, the dictionary's treatment of even common usages in science is hesitant at best, as may be told from the article on "anti-particle" in the new edition. Second, it is too dependent on British sources. When everything had to be done by hand, there may have been no choice. Now there is a chance to make it into an English dictionary in the full sense. That should not be neglected. □

# Shake-up in store for Britain's biologists

- New organization eliminates fragmentation
- Single funding source proposed for all biology

## London

THE biological sciences in Britain's universities are underfunded, short of manpower, and teaching and research are "undesirably fragmented", according to a committee set up by the Universities Grants Committee (UGC) to advise on how academic biology could be 'rationalized'. The results of the review have been shrouded in secrecy until now, and there is likely to be heated debate about the conclusions at a meeting this week of the heads of Britain's biology departments.

Similar reviews of chemistry and physics last year concluded that to maintain quality with the limited funds available resources should be concentrated in large departments—those with at least 20 full-time staff. But the biology committee concludes that resources for biology must expand. Professor Richard Southwood of the University of Oxford, who chaired the UGC committee, says biology will be the "leading science of the twenty-first century". He complains that biology has always been underfunded because when interest in the subject increased in the 1970s, a period of university expansion had ended and there were increasing constraints on growth. An injection of funds is now necessary to compensate for this, he says.

The committee says that the main problem facing biology is the fragmentation of teaching, research and of the funding mechanisms. To eliminate fragmentation in support, the report recommends money for biology be provided from one research council instead of the current four. Most support for biology is now channelled through the Science and Engineering Council (SERC). But to avoid competition with the expensive demands of the physical sciences and engineering, the responsibility for biology should not be vested in SERC but in a new biological sciences council.

For research and teaching, the report recommends that all biology departments be grouped into a single organization, within which there should be two distinct subgroups. One, termed M, would focus on molecular sciences, including biophysics, biotechnology, physical biochemistry and molecular genetics. The other, called B, would focus on traditional areas of biology such as physiology, ecology, entomology and evolutionary studies. Most biologists do not dispute that more integration of their departments is desirable, but the M/B

idea is nonetheless controversial. To ensure that all facets of biology are covered in each department, the committee says there would need to be 5 research groups each with at least 4 members of staff, so each department would contain at least 20 members of staff.

Meeting the requirements of size and covering the defined areas at the same time would require "an enormous amount of restructuring" in universities, said Professor Christopher Arme of the University of Keele, though he agrees that larger departments than exist at present would be desirable. Others have a different view. Professor David Cove of the University of Leeds argues that small departments can be more efficient than large ones, and a greater amount of personal attention to students is rewarded with a better quality of student.

Southwood stresses that closure of any biology department would be short-sighted. The predicted demographic dip in the 1990s leading to fewer young people of university age is a misleading indicator of future demand. There is likely to be an acute shortage of suitable personnel for posts in biology in universities and research-council institutes in the future, says the report. It supports the schemes of the UGC and the Royal Society to tackle this problem, but warns against central planning in relation to subject and place of study.

One of the concerns of the committee is that traditional areas of biology are increasingly being neglected, and it believes that there should be an equal number of M and B departments. Undergraduates would take a three-year degree course in one department, but should have a basic understanding of the biology studied by the other groups. The committee also warns against central planning in distribution of research funds. It is impossible for committees to identify topics that will lead to significant advances in the future, and best value for money will be obtained by research funds allocated by heads of departments. A spectrum of mechanisms for distributing funds should be pyramidal, with a base of many project grants and relatively few large interdisciplinary research centres. Though the government is now considering separating the allocation of funds for teaching and research in Britain, the committee warns against taking any responsibility for research funds away from the universities.

**Christine McGourty**

## NEWS IN BRIEF

### Alaskan oil spill

#### Washington

THE oil tanker *Exxon Valdez* last week struck a reef and spilled some 11 million gallons of crude oil into Prince William sound off the south coast of Alaska. The spill is being called the worst in North American history. The tanker had just left the port of Valdez, the south terminus of the Trans-Alaska Pipeline and was carrying 53 million gallons of oil. Clean-up crews are trying to contain the spill which threatens severe disruption to the local ecosystem. J.P.

### Better health ahead

#### Sydney

A NATIONAL strategy for Aboriginal health, released at the annual meeting of health ministers last week, is the first attempt in ten years to deal with the health problems of that population. Australia's 228,000 Aborigines suffer from poorer health and a lower life-expectancy than the general population. The World Health Organisation said last year that Aborigines have the worst reported standard of health of any indigenous people. The government's strategy includes plans for upgrading health services, providing more health training and educational programmes, and transferring basic Aboriginal health services from the state governments to the Aboriginal health community. The recommendations, if implemented, are expected to cost A\$417 million. T.E.

### Hair-raising scheme

#### New Delhi

HUMAN hair is one of India's resources that has heretofore gone to waste. But an Indian and a Japanese company have now formed a partnership to turn what was discarded into profit.

Hair is a potentially profitable source of amino acids. Under the new arrangement, Union Bros of Japan will supply expertise, equipment and part of the finance for a \$10-million processing plant being set up at Pondicherry in south India. The plant will consume 1,200 tonnes of human hair a year, turning it into 80 tonnes of L-cysteine, 24 tonnes of L-tyrosine and 1,400 tonnes of dry powder containing as many as 19 amino acids. The products are extensively used in the pharmaceutical, food-processing and cosmetic industries. The company expects to make 25 per cent profit and Japan will purchase the entire output for 8 years. Human hair has become an important raw material for amino acids after whaling restrictions made that source scarce. Apart from barbers' shops, the biggest source of human hair in India is the temples, where Hindus shave their heads as a religious custom. One temple in Tirupati Inandhra Pradesh collects 4,000 tonnes of hair a year. K.S.J.



## Cold results from Utah

### Washington

AN announcement last week by the University of Utah in Salt Lake City that physicists there had achieved nuclear fusion in a test tube at room temperature has been followed by a flurry of speculation about not only the strange science involved but also the peculiar publicity given to it.

The technique involves nothing more complicated than electrochemistry. Current is passed through a palladium electrode immersed in a solution of various salts in deuterated water. Over a period of many hours, deuterium atoms are absorbed by the palladium lattice in such numbers that some of them fuse, forming tritium or helium-3, simply because of their proximity. A return of four watts of heat from one watt of electricity was reportedly obtained when current was passed for a total of 100 hours.

According to James Brophy, vice president for research at the University of Utah, the decision to announce the work in preliminary form at a press conference last Thursday, before a paper had been accepted by a scientific journal, was a response to press enquiries. But the university press office had previously contacted several science reporters and encouraged them to attend.

A statement by the University of Utah that results "will appear in the scientific literature in May", coupled with a report in last Thursday's *Wall Street Journal* that papers had been submitted to *Nature*, provoked numerous telephone calls to *Nature*'s Washington office enquiring whether we had accepted or were about to publish one or more papers. (The answer is no.) Meanwhile, a group at Brigham Young University in Provo, Utah, led by Steven E. Jones, has refused to say whether they have obtained similar results.

Enthusiasm over the claim has been tempered with a good deal of scepticism, not only due to the lack so far of a detailed account but also because the history of fusion research has been regularly punctuated by 'breakthroughs'. The new claim bears some similarity to muon-catalysed fusion, in which the substitution of a muon for an electron in heavy hydrogen shrinks the size of the molecule enough for spontaneous fusion of the two deuterons to occur. Reasonable rates have been achieved in small-scale experiments, but as an energy-producing method muon fusion is no nearer success than any other technique. The University of Utah has applied for a patent on its technique, but Jones says only "don't sell your oil stocks yet". □

## Academy starts over again

### Moscow

AT the general meeting last week of the Academy of Sciences of the USSR, only 8 of 20 delegates were elected to the All-Union Congress, from which delegates will ultimately be elected to the Supreme Soviet of the USSR. The Academy's election process will now have to begin again.

The normal election procedure was complicated by the fact that after an extended plenum of the leading bodies of the Academy on 18 January, just 23 candidates for the 20 delegates out of 130 candidates proposed by research teams at academic institutions were chosen (see *Nature* 337, 293; 1989). The failure to nominate those scientists receiving the greatest popular support—including Academicians Andrei Sakharov and Roald Sagdeev—evoked particular discontent. A lobby was set up for the democratic election of People's Deputies from the Academy of Sciences of the USSR, which held a protest meeting in front of the Academy Praesidium's building in Moscow, and organized what amounted to a boycott of the election of the nominated candidates.

According to the law, additional elections have to be held to fill the remaining 12 delegates within the next two months. The new election process began during last week's general meeting, when procedures and potential candidates were discussed. Describing the outcome of the elections in his speech at the conference, Sakharov emphasized that emotions were running so high not because "Sakharov was not nominated, but because the role of the research institutions was ignored".

Academician G.I. Marchuk, president of the Academy, explained the current election phenomenon by saying that "all of Soviet society, including the Academy, is going through a historical moment—the transition to people's power has started in the USSR in earnest. The main point is that the elected deputies will represent not just the interests of the Academy but also and above all the interests of the social development of the nation as a whole."

Voting procedures now call for the 15 unsuccessful candidates to be asked to agree to run again. In addition, many scientists from the previous list suggested by various institutes, as well as new names, were mentioned. Judging by the response of the audience, the following scientists should be on the list of new candidates: Academician Stanislav Shatalin, an economist; Academician Vitaly Ginsburg, a physicist; and the economist Nikolai Shimeley. New elections will probably be held next month.

The conference decided that new candidates will be nominated by the Praesidium of the Academy of Sciences of the USSR. This responsibility has now been vested in it after the extended plenum of the leading bodies of the Academy failed to rise to the occasion. The members of the former election commission of the Academy of Sciences on the election of People's Deputies have resigned, and a new commission will be formed. The date for a new election conference has not yet been fixed. Importantly, the voting will be by secret ballot at meetings of research associates of academic institutes.

**Yuri Kanin**  
*Novosti*

### WEATHER FORECASTING

## Precipitation precipitates complaints

### Sydney

AN insufficient number of weather stations are being blamed for Australian weather forecasters' failure to provide early warning of torrential rains that flooded parts of central Australia, causing significant stock losses. Rains have been particularly heavy in Australia this year. At Broken Hill, in western New South Wales, 180 millimetres of rain fell in a slightly more than 24 hours, the heaviest fall that had been recorded since 1973. Storm warnings by the Department of Meteorology came on the morning of the fall, too late for some farmers.

A reduction in staff salaries and productivity costs means much of Australia's weather remains undetected. "We have a country the size of the United States with one tenth its population. The quality of

our meteorological services is related to the observational networks we can afford", said Bill Downey, assistant executive director for the Australian Bureau of Meteorology.

Peter Noar, assistant director of weather services, acknowledges that a full network of a couple of hundred observation stations is needed. Some help may come from plans to expand land-based automatic weather stations and improve the network for aviation forecasting.

But the problems at Broken Hill are threatening to get worse before they get better. The Civil Aviation Authority airport observation station may close, forcing the Bureau of Meteorology to try to come up with sufficient funds to open its own station in Broken Hill.

Tania Ewing

## SPACE STATION

## REMOTE SENSING

# Banging the Freedom drum

## Washington

NATIONAL Aeronautics and Space Administration (NASA) officials like to say that no matter what objectives are chosen by the United States for space activities in the twenty-first century, "all roads begin with the space station Freedom". But as NASA pleads its case before a budget-conscious Congress, Freedom's \$16,800-million price tag practically guarantees that its own road into space will be a rocky one.

Now set to be deployed in the mid-1990s, the space-station programme is building momentum. Last September, Canada and the European Space Agency signed formal participation agreements (see *Nature* 335, 480; 1988), and earlier this month Japan signed a memorandum of understanding. Altogether, NASA's foreign partners will contribute \$8,000 million to the project.

President George Bush is an enthusiastic proponent of the space station. Nervous about the cost, Congress last year appropriated \$900 million for the space station, but ordered \$515 million to be held back until 15 May this year so the new president could decide whether to proceed. President Bush has already indicated that the money will be released for the space station.

This year, NASA is requesting \$2,050 million for its space-station activities. Three-quarters of that money will go to the four aerospace companies with which NASA signed contracts last September to build major elements of the station. Boeing is developing the crew living quarters and logistic elements; McDonnell Douglas is pursuing the central truss structure and resource nodes; General Electric is developing the polar platform and attached payload accommodations on the main station; and Rocketdyne is developing the power systems.

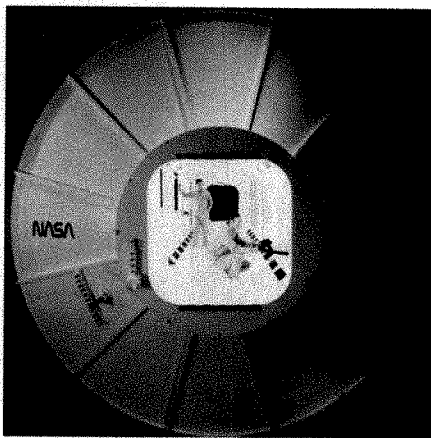
For its part, Congress is enthusiastic about the idea of the space station, but budgetary concerns make any new and expensive project vulnerable. The word around Congress is that as much as \$1,000 million may be cut from NASA's total 1990 budget request of \$13,300 million, and some of that is bound to come from the space station.

But space-station officials are already feeling pinched, and have warned that further cuts could be devastating. NASA has not been able to find funds for nearly a third of the 2,644 posts authorized for the programme. At a hearing on 16 March before the Senate Commerce, Science and Transportation Subcommittee on Science, Technology and Space, NASA Associate Administrator for the Office of Space Station James Odom

testified that "virtually any cut would do major hurt, and a significant cut would be tantamount to killing the programme". Odom also warned that backing out on NASA's commitments to its foreign partners would also have devastating effects.

Launching the space station is planned to take 20 space-shuttle flights, the first of which is now scheduled for 1995. Human occupancy should be possible after the fourth shuttle flight, and a permanent crew can stay on board after flight 13.

The space station's attached science payloads — now likely to include a gamma-ray/cosmic-ray focusing device called Astromag and a cosmic dust sampler — are intended for flight 7, an early



Installing equipment in Boeing's mock-up of Freedom's living quarters.

flight that supporters say is indicative of NASA's commitment to doing science on board the space station. The launch schedule for the flights is based on current shuttle lift capabilities, and a new advanced solid rocket motor, once it becomes available, could speed up the process.

A decision to move forward with shuttle-C, a cargo-only version of the shuttle devised as an alternative option, would also be a boon for the space station, but so far the administration has shown little support for this plan.

Coordinating the development of hardware with that of scientific instruments so that both will be ready to fly on time will be a tricky business. As an example, General Electric has already begun work on the polar platform, but funding for the platform instruments, which have already been selected, will not be formally requested until the 1991 fiscal year. Last-minute modifications to spacecraft hardware to accommodate instruments can be extremely expensive, and can cause delays.

Although NASA has agreed with the

## On-again, off-again Landsats on again

### Washington

LANDSATS 4 and 5 are back in business — for the moment. The Earth Observation Satellite Company (EOSAT) announced on 15 March that the shut down order issued by the National Oceanic and Atmospheric Administration (NOAA) had been rescinded.

EOSAT, which operates the two Earth remote sensing satellites for NOAA, had begun shut down procedures that would have terminated Landsat operations at the end of the month (see *Nature* 338, 194; 16 March 1989).

Details of the financial rescue package have not been revealed, but EOSAT says it costs \$9.4 million to operate the two Landsat spacecraft. NOAA officials will only say that the reprieve will last at least until the Bush Administration completes a review of the programme now under way.

The rescue effort was coordinated by the new National Space Council chaired by Vice-President Dan Quayle. Members of Congress have reported that their offices have been inundated by letters asking that money be spent to keep the Landsats alive.

Joseph Palca

White House Office of Management and Budget to cap spending on the space station at \$16,800 million, several elements of the station, such as operations costs, facilities, launch costs, communications support and several new features including an emergency crew escape vehicle, are not covered by the cap. Other programmes at NASA have been cut back in order to provide adequate money for necessary space-station development and activities.

There has long been tension between space scientists and those favouring manned space activities. Station chief scientist John Bartoe acknowledges that selling the station solely as a tool for science is untenable, but that it can be useful for scientists once they learn its capabilities. It seems likely that the successor to James Fletcher, who has announced his resignation as NASA administrator, will continue the strong support NASA has traditionally provided to the manned space programme.

Proponents of the space station are trying to secure the high ground in their efforts on behalf of the space station. At the Senate hearing earlier this month, Senator Al Gore (Democrat, Tennessee) set the tone for the coming Congressional debate that will make opponents of the space station squirm: "The choice facing us this year is not just between moving ahead with the space station or not, but between continuing leadership in space and abdication and decline."

Joseph Palca

## Transgenic sticky issues

### Washington

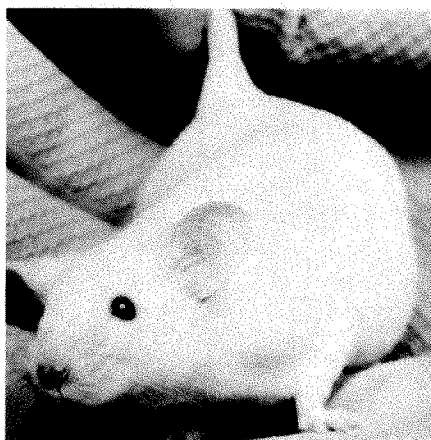
ANIMAL rights, the release of genetically engineered organisms into the environment, and the effects of large agribusiness companies on the farming industry are the real issues behind the debate over the patenting of animals, according to a report\* released last week by the US Congress's Office of Technology Assessment (OTA). Existing regulations can be adapted to address most of the practical considerations of animal patenting, such as whether farmers should pay royalty fees for breeding patented livestock. But the ethical question of whether or not transgenic animals should be subject to patents is a question that may need further clarification, OTA says.

Congress has heard both sides of the debate since the patent office declared in early 1987 that it would not reject patent applications on the sole basis that the invention for which the patent was being sought was an animal. The granting of the first patent for a higher life form last year — to Harvard University, for a transgenic mouse containing human on-

cogenes (see *Nature* 332, 668; 1988) — heated up the exchange between patent proponents and those who asserted that animal patenting would lead to animal suffering and higher costs for farmers.

But attempts in the past two years to pass legislation to halt the patenting of additional animals until the ramifications could be worked out — and specifically to exempt researchers and farmers from royalty payment requirements — have floundered. The Animal Legal Defense Fund has also lost the first round in a court battle to overturn the patent office's decision to patent animals.

In the meantime, 44 patent applications covering animals have piled up at the patent office, and the exclusive



Du Pont's OncoMouse, the only patented animal so far.

licensee of Harvard's patent, Du Pont, is taking orders for its \$50-a-piece OncoMouse, which it plans to begin shipping in May. Other companies are not waiting for patent protection to develop profit-making genetically engineered animals. Integrated Genetics has formed a collaboration with Tufts University to develop herds of cows capable of excreting human proteins in their milk, and the company Transgenic Sciences has just been formed, and intends to develop animal breeds which produce valuable pharmaceuticals.

Representative Robert Kastenmeier (Democrat, Wisconsin) last week reintroduced bills he put before Congress last year that would create a transgenic animal advisory committee within the Department of Agriculture which would authorize permits to field-test transgenic animals. Kastenmeier's bills also specifically exempt farmers and researchers from paying royalties on patented animals. Kastenmeier says he hopes biotechnology companies and opponents of animal patenting can work out their differences, an acceptable compromise, but

\*New Developments in Biotechnology — Patenting Life, Office of Technology Assessment, Washington DC, 1989.

## New carp park ahead

### Washington

THE biotechnology review board of the United States Department of Agriculture last week approved the first field-test involving a genetically-engineered fish. Researchers at Auburn University in Alabama have been given permission to introduce carp which contain trout growth hormone genes into a test pond at the university.

The carp were developed through a collaboration between researchers at Auburn, and at Johns Hopkins University in Maryland. Carp containing the trout genes have been shown in the laboratory to grow significantly larger than normal carp, a finding that could have commercial significance for fish farming.

The carp will be kept in a contained pond, with a series of barriers to prevent their escape into open water. As an added precaution, a mechanism has been developed to introduce poison into the water should a fish manage to get beyond one of the barriers.

The Auburn carp is the first transgenic animal to be approved for release into the environment by the Agricultural Biotechnology Research Advisory Committee, which has been charged with developing guidelines for reviewing field-test applications from researchers working under Department of Agriculture grants. The finishing touches are now being put to the committee's guidelines, which should be published in the Federal Register for public comment within the next two months.

The Department of Agriculture is opposed to legislation introduced last week by Representative Robert Kastenmeier (Democrat, Wisconsin) which would require it to set up a separate review board to grant formal permits for field-tests of transgenic animals. Daniel Jones from the Department's Office of Agricultural Biotechnology says that laws already on the books should cover transgenics, with some "fine-tuning" from the new guidelines. Jones says the guidelines will parallel those covering recombinant molecules developed by the National Institutes of Health: they will be compulsory for academic grantees, and industrial researchers are expected to adhere to them voluntarily.

Carol Ezzell

that he will support a halt to animal patenting if the means to address the sticky issues surrounding such patents is not put in place.

The Congressmen who proposed a moratorium on the patenting of animals last year, Representative Charlie Rose (Democrat, North Carolina) and Senator Mark Hatfield (Republican, Oregon), have no plans to introduce their bills again this year.

Carol Ezzell

### CHEMISTRY

## NERC finds British chemistry ailing

### London

CHEMISTRY in Britain is suffering from insufficient investment, inadequate equipment and poor dissemination within the research community of information on facilities available. Those are the main conclusions of a committee set up by the Natural Environment Research Council (NERC) to review the health of chemistry. The report, published this week, concludes that facilities are outdated, and most researchers feel they are lagging far behind their counterparts in other European countries and in the United States. The committee says that because much of the equipment in universities and NERC institutes is old, slow and insensitive, the quality and quantity of data is suffering. This in turn may be leading to the loss of commissioned research contracts.

To remain competitive, Britain must have well-equipped, state-of-the-art laboratories, and this will require a substantial programme of investment in chemical equipment, says the committee. Its report points out that although the cost of equipment is increasing faster than the rate of inflation, its speed and sensitivity are increasing even more rapidly, so the cost-effectiveness of new equipment is improving in real terms. At present, even the equipment available is used insufficiently because of severe constraints on travel and subsistence budgets.

Christine McGourty



## CHERNOBYL

# Soviet data made public

London

RADIOACTIVE contamination from Chernobyl is still "an acute technical and social problem" likely to persist for a considerable time, according to Dr Yuri Izrael, chairman of the State Committee for Hydrometeorology, writing in *Pravda* last week. In a full-page article, he presented the first figures and maps for the whole area of the Soviet Union affected, comprehensively extending the data for Byelorussia given at a public meeting in Minsk last month (see *Nature* 337, 683; 28 February 1989).

The release of these data is an important development, a departure from the policy described by Kiev radio earlier this month as the "tranquillizing" of the population. Contrary to the impression conveyed at Minsk that the collection of data had only just been completed, Izrael also says that the collection of data began on 26 April 1986, the day after the accident, and that a generalized map covering a 60- by 40-km region showing contours of gamma-radiation was available on 10 May that year, in time to guide the evacuation of people from contaminated zones.

In the first few days after the accident, Izrael says, the first 2 milliroentgen per hour contour enclosed about 200,000 km<sup>2</sup> of Soviet territory, with islands of contamination as far away as the Kola Peninsula in the far north, Ivano-Frankovsk in the south-west and the Caucasus in the south-east.

"Massive" testing of soil samples began in June 1986, when exceptionally high proportions of the long-lived isotopes caesium-134 and caesium-137 were found. But Izrael says that the lifetime exposure limit of 35 rem of external radiation was applied to the caesium-

contaminated areas only in November 1988, leading to the evacuation of a further 20 villages in Byelorussia.

Pollution of rivers and reservoirs remained "within the limits of the agreed norms" in the months after the accident, according to Izrael, who says that total beta-activity amounted to between 1 and  $6 \times 10^{-9}$  curie per litre during the first two months. (The safety limit was  $10^{-8}$  curie per litre.) During the spring floods of 1987, Izrael says, even the rivers with radiation 'hotspots' in their catchment areas were well below the limit.

Further aerial and ground surveys carried out in 1988 are said to have revealed substantial changes of the contours. At present, Izrael says, for caesium-137 the 15 curie per km<sup>2</sup> contour encloses some 10,000 km<sup>2</sup>, of which some 3,500 km<sup>2</sup> have been evacuated, while the remainder has a population of 230,000 people. In the town of Slatytych, built for Chernobyl workers after the accident, radiation levels (0.013 to 0.03 milliroentgen per hour) are close to the natural background, although there are some hotspots in the surrounding forests which nevertheless do not exceed the safety limits.

Although Izrael's sympathy for the new anti-nuclear groups in the Soviet Union is clearly muted, he concedes that the decision to close the two Armenian reactors (at Yerevan, one last month and one this) was "quite understandable". But he concludes that, with the threat of the greenhouse effect, nuclear generation is the "most promising" form of energy. The lessons of Chernobyl, he says, imply that the operation of existing nuclear reactors is "many times", and of future stations, "many orders of magnitude", safer than in the past. **Vera Rich**

## ABORTION

# French drug under attack

Paris

THE controversial abortion pill RU-486, which was approved by the French Health Ministry for use in abortion clinics last year amid angry opposition, is again under attack (see *Nature* 336, 4; 1988). Dr John Willke, of the US National Right to Life Coalition, is in Europe for the annual meeting of the International Federation for the Right to Life in Brussels. Before the meeting he met the medical research director of Roussel-UCLAF, the company that makes the drug, and suggested that the company's products could be boycotted in the United States if the drug is not withdrawn.

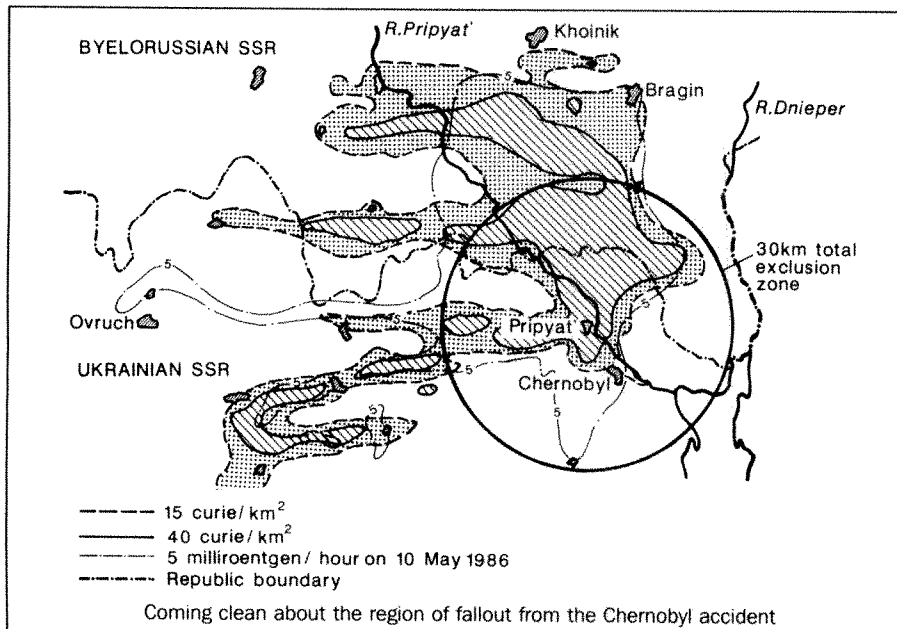
France is the only country to have legalized the use of RU-486 for abortion, and the drug is administered only under strict medical supervision and only in association with prostaglandins. In October last year, Roussel-UCLAF suspended sales of the drug after some of its senior employees and their families received anonymous death threats. It was only after government intervention that the drug was again put on the market.

Roussel-UCLAF, a subsidiary of the pharmaceuticals giant Hoechst, declined to comment on the meeting with Willke. A spokesman said that RU-486 is only a new alternative to surgery and its withdrawal would have no effect on the number of abortions carried out. In Washington, the National Right to Life Coalition also declined to comment on possible boycott action while Willke is still in Europe.

Right to Life is one of the most vocal and powerful minority lobbies in the United States, capable of assembling thousands of people at rallies to protest against abortion. Any decision to boycott Hoechst products would therefore be taken seriously by the company, even though it has no plans to introduce RU-486 in the United States.

Although it is illegal for federal laboratories to carry out research on abortifacients, the potential contraceptive properties of RU-486 are being studied. At low doses, the drug is thought to impair the implantation of a fertilized ovum in the uterus lining. Since implantation is a criterion for pregnancy, such a function would technically be 'contraceptive' and not 'abortifacient'.

There is no national legislation on abortion in the United States; only a Supreme Court ruling makes it illegal for states to outlaw abortion. But a test case currently before the Supreme Court has challenged this ruling. The judgement, which could come in the next month, will stir emotions in the anti-abortion lobby, whatever the outcome. **Peter Coles**





# Hope for nuclear industry?

## Boston

THE US Nuclear Regulatory Commission (NRC) is expected soon to authorize sweeping regulatory changes that will shorten the licensing procedure for nuclear plants. The new procedure eliminates the requirement for a separate operating licence for newly constructed reactors, thereby limiting the opportunity for public intervention once a plant has been built. The five-member nuclear commission is expected to vote on the licensing overhaul as early as this week, and the measure is said to be almost sure to win approval.

Renewed interest in nuclear power and frustration over the experiences at the Shoreham and Seabrook plants —

## INTERNATIONAL SCIENCE

# Non-aligned line-up for new technology

## New Delhi

A centre for science and technology of non-aligned and other developing countries was formally established in New Delhi last week after 31 countries signed the centre's statute. Joint research programmes and exchange of experts are some of its objectives, but the centre primarily seeks to pool existing scientific resources, technological and consultancy capabilities in member countries for their common benefit. The centre will be financed by contributions from each member country, fixed at \$15,000 per year. The initial 31 countries are from the Indian subcontinent, Africa and Eastern Europe, but China is not a member. As the host, India has offered an extra \$1.1 million for office building, equipment and transportation. The first-year budget of the centre is \$1.6 million. It will have an Indian director but other key posts are to be filled by people from other member countries.

Computer software, biotechnology, renewable sources of energy telecommunications and new materials are among the areas from which about 30 specific collaborative programmes have been identified. Project expenses will be met by participating countries; the centre will play only a catalytic and coordinating role, although there will be money for pilot plants or infrastructure development.

The centre's first task is to prepare a directory of science and technology institutions, and a list of transferable technologies already available in member states. There are proposals for a common gene bank, a shared database on science and technology and a consortium for technology promotion within the non-aligned club.

K.S. Jayaraman

both now built but not operating — have propelled consideration of the new rules. Proponents of the changes argue that the current licensing procedures are unnecessarily cumbersome and expensive to follow.

If adopted, supporters say the new rules will shorten the time needed to begin operating a new plant, simplify the site-selection process and bring standardization in reactor design. In addition, advocates believe that the changes would be an important symbolic incentive to the nuclear industry and to poten-

ess. The new rules would not affect any existing plants (licensed or otherwise).

The idea of revising the licensing procedure for nuclear power plants has been considered for several years, but has usually been discussed as a matter to be dealt with by Congress. Although members of the nuclear regulatory commission and the nuclear industry have urged Congressional action to simplify the licensing procedure, Congress has so far failed to take up the matter. According to one inside observer, the commission "finally got fed up with Congress and decided to go out and change the licensing procedure on their own".

But Ken Bossong, director of the Crit-



The way things were: work in progress on the Palo Verde nuclear generating station

tial financial backers of nuclear power.

But critics argue that the proposed changes are illegal and unsound, and that they are a "blatant effort" to limit public involvement in the licensing process. If passed, the rule changes are sure to face a legal challenge. According to several sources, anti-nuclear groups are meeting currently to decide "who will take the lead" in the legal challenge.

Under the proposed licensing process, only one licence will be given by the nuclear regulatory commission at the construction phase of a new nuclear reactor which would be a combined "construction and provisional operation" licence. At some point shortly before construction of a plant was complete, the utility would seek approval to 'implement' its operating licence.

In other changes would allow utilities to obtain "early site permits" up to 20 years before building a plant. Public disputes over the suitability of the location for the reactor would have to be settled at this early stage. The nuclear regulatory commission would also "pre-approve" several standardized nuclear reactor designs. By using one of these designs, utility would be exempt from part of the present public review proc-

ical Mass Energy Project, likens the proposed changes to awarding a university students a degrees "based only on their first year of school". He and other critics contend that the proposed changes "decrease if not eliminate" the possibility to consider flaws or fraud in the construction process, or changes in the site situation — such as roadways, population changes, hospitals or schools — that may occur during the 20 year period after the site has been approved.

In addition, Bossong and others say that the new rules preclude evaluating the financial ability of the utility to operate the plant once construction is finished. Despite the controversy already raging around the rule changes, there is little certainty over how they might affect the beleaguered nuclear industry.

Although they ought to make the construction and licensing process simpler and more concise, several observers note that the financial community is greeting the proposed changes warily. As Bossong puts it, "the industry still has to grapple with widespread public opposition to nuclear power, lack of nuclear waste facilities and economics that are not competitive with other sources of energy".

**Seth Shulman**



## GREEN POLITICS

# New pragmatism pays off

## Munich

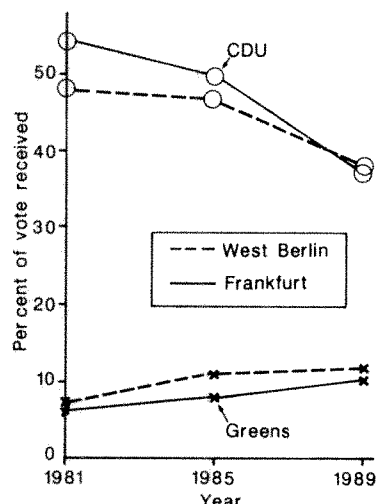
THE Greens are on the rise again in West Germany. For the first time in two years, a *Land* (state) government including the Green party was formed in West Berlin on 16 March, pointing the way towards more Green political power. Ten days earlier, national party members elected an executive committee (*Vorstand*) intent on forming a coalition with the Social Democrats (SPD) in the *Bundestag* after the 1990 election. The possibility of an SPD/Green coalition is stronger than ever in the aftermath of heavy losses for the conservative Christian Democratic Union (CDU) in recent elections.

Even as their political fortunes are waxing, the Greens are taking a less utopian view toward ridding society of what they see as its technological evils. A survey of Green politicians in Bonn and elsewhere reveals a more pragmatic attitude towards divisive issues such as genetic engineering, nuclear research and space exploration. Once advocates of banning everything to do with technology, ten years of political experience has tempered Green extremism.

The Greens shared power once before in a stormy coalition with the SPD in the *Land* of Hesse from 1985 to 1987. But a conservative coalition led by the CDU took over, in part because of governmental paralysis on nuclear issues. They faded from view after that, plagued by internal struggles. The election of a pragmatic leadership at the Green party conference in early March reveals the new direction of the party. The three executive committee spokespersons elected—Verena Krieger of Witten, Ruth Hammerbacher of Osnabrück and Ralf Fücks of Bremen—were careful to avoid the kind of name-calling that was so destructive two years ago. The election, and the

decision of West Berlin's Greens to form a coalition with the SPD, reflects the wishes of Green voters to see their leaders take action, even if it means compromise.

Because of its unique status, the Greens' success in West Berlin is hard to extrapolate to the rest of the country.



Small but steady progress of Greens.

The *Alternative Liste* (AL), a local Green party, had always been strong in some areas of West Berlin, which has a large population of draft-resisters and dropouts from society. After weeks of debate, the SPD and AL reached a compromise on governing the city.

But the results of community elections in Hesse on 12 March suggest that the Berlin result was not a fluke. Green support surged all over Hesse and helped topple a CDU government in West Germany's financial capital Frankfurt. A new political constellation seems to be forming which is widely seen as a threat to the re-election prospects of Helmut

Kohl's conservative coalition in 1990. According to a poll taken by the magazine *Spiegel* in February, Kohl's CDU and its Bavarian sister party CSU had reached a two-year low in popularity.

The compromise worked out between SPD and AL in West Berlin gives some clue as to what sort of science policy to expect in a national SPD/Green coalition. For example, the AL yielded on initial demands to stop construction of a nearly completed nuclear research reactor at the Hahn-Meitner Institute. Receiving a final licence for the reactor from the new environment senator, a member of the AL party, will be difficult, but the SPD is expected to ensure that it will be possible.

National Green politicians are quick to assure that they would not shut down research institutions. "It would be crazy to forbid nuclear physics research", says *Bundestag* member Otto Schily, "just because the atomic bomb is a danger to mankind". If the Greens come to power, says Schily, the freedom of research that is guaranteed in the West German constitution (*Grundgesetz*) "must not be touched". Schily belongs to the more 'realistic' wing of the Greens.

The most visible feature of the West German research scene that would be affected by Green involvement in government is space policy. West Germany's active collaboration in European Space Agency projects has been supported by a publicity-conscious government that seeks as much prestige as possible for its space programme.

Fücks criticized the space programme because of its ties to the military. Given all the ecological goals the Greens have in mind, it would be hard to continue to invest in expensive programmes such as manned space flight. Despite the criticism, it is unthinkable that a new West German government would renege on international contracts signed by its predecessors. Schily categorically excludes this possibility. The Greens still advocate a shutdown of all nuclear plants, and their views are not far from those of the general public in West Germany. Nearly 80 per cent of the population would prefer an immediate or gradual shutdown, according to the *Spiegel* survey.

Like nuclear power, genetic engineering is an issue that calls for sweeping condemnations from Green politicians. But the current leaders have begun to break from their predecessors in admitting some practical uses for molecular biology, such as the development of AIDS treatments.

Maintaining their political momentum going into the long series of *Land* elections in 1990, culminated by the *Bundestag* election, will be difficult. But recent events have ensured that the Greens will not just go away. **Steven Dickman**

## ENVIRONMENTAL PROTECTION

## Compromise reached on toxic waste

### Paris

DELEGATES from 116 nations meeting last week in Basel, Switzerland, under the aegis of the United Nations Environment Programme, failed to reach unanimous agreement on rules governing dumping of toxic waste. Only 34 nations signed a treaty, which had taken 18 months to draft, but 105 did sign a final compromise document. The United States, Britain and the Soviet Union were among those calling for more discussion before signing a treaty.

Some developing nations wanted a total ban on the export of toxic waste because, they argue, they are easy prey for industrial companies seeking to dispose of waste away from their own back yard. But for most European and other industrialized nations, waste disposal is an acceptable

commercial enterprise and a ban would be out of the question.

The Organisation for African Unity sought powers to search ships in coastal waters for clandestine cargoes of hazardous substances, but this is against international maritime law. As a compromise, the summit drafted an agreement by which signatories undertake to export waste only to countries having sufficient facilities and expertise to deal with it and only following written confirmation by both the exporter and the importer. Signatories also agree to reimport waste rejected by another country, but they will not be obliged to accept responsibility for accidents arising from waste dumped before the new regulations come into force.

Peter Coles



## Distinguishing fraud from error

SIR—There is a danger in the controversy over fraud in science of merging the concepts of fraud and error. The call for an audit of scientific papers for error is a symptom of this trend. Fraud such as fabricating data or publishing the work of others as one's own is of course serious, particularly when it involves assessment of drugs and other medical treatments where lives are at stake. But error is an inevitable part of science. This fundamental point is being missed in the current debate. Scientists struggle to express their ideas, struggle with recalcitrant organisms, use advanced laboratory techniques that they barely understand, and study systems so complex that many hypotheses may seem to fit the data. This is not the orderly process that some imagine science to be. One does not go into a laboratory and order a cure for heart disease and get a guaranteed result. Results in science are never guaranteed and are never free from error. Newton, Einstein and Darwin were all wrong in some respects, even though they are the standard by which we judge genius itself.

Science is a complex and subjective process, one man's enthusiastic promotion of an hypothesis may be another man's fraud. The history of science is full of individuals who turned out to be right but who, by the strict criteria proposed by some today, could be attacked for fraud. In the laboratory notebooks of Millikan's oil-drop experiments, it can be seen that he rejected many trials because his judgement told him that something was wrong. In today's climate he could be accused of blatant manipulation of the data, that is, fraud. The 'discoverer' of N-rays had bad technique and practised self-deception, but there was no fraud. Was Columbus guilty of scientific misconduct for his self-delusion about the circumference of the Earth? Kepler defended himself in his search for the "Music of the Spheres".

Some of the charges levelled in recently publicized cases include misclassification of control subjects, poor laboratory procedure (Benveniste) and complex issues of scientific method (Baltimore). These are real scientific issues, but not causes for hand-wringing and raised eyebrows, much less for congressional scrutiny.

Who ever said that when you open a journal you are reading a message carved in stone by some higher being? Every time one reads the scientific literature, it is necessary to be wary and to compare, test and evaluate methods, data and statistical methods. At no point do we have such a grasp on the truth, statistical, experimental or otherwise, that a panel can be certified as error hunters. Reviewers of a

paper are themselves often wrong (the original theory of continental drift was met by howls of laughter from 'respected' scientists). Who will review the error hunters? Who is qualified to punish whom?

So, is there error? Of course there is. But we should also note that every building built has flaws and half of them are ugly, every economic policy ever formulated is imperfect. We can only ask for perfection when the correct answer is known (for example, on the factory assembly line), but if the answer is known then it isn't science.

CRAIG LOEHLE

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## Defence research

SIR—You report (*Nature* 336, 610; 1988) that the veterinary school at this university is being supported in a research project on the survival of bacterial pathogens in aerosols by the Ministry of Defence (MoD) Chemical Defence Establishment. The project is of evident relevance to biological warfare, if for no other reason than that proposals must have 'defence relevance' to qualify for MoD support. Whether or not the ministry's interests in biological warfare are legitimate, we believe that they are not appropriate to a veterinary school.

Unlike the research councils, MoD has no remit to improve animal health and welfare nor a broad peer-group-defined strategy. The medical and veterinary benefits of the Bristol project are merely a spin-off of its defence interests.

While research support by the research councils appears to be increasingly inadequate, MoD support of research in British universities and higher education establishments has increased by 60 per cent since 1984-85 (from £10 million to £16 million). Consequently there is a danger that scientists are relying increasingly, as at Bristol, on support from government or from commercial organizations with narrow research objectives and a strong interest in confidentiality, even secrecy. We believe that such support undermines the integrity and openness with which research can be conducted.

Clearly, the freedom with which a project is formulated is compromised when it has to conform to concerns of a sponsor (for example, defence or financial profit) that are completely irrelevant to the purpose of the research (for example, the improved health of livestock). The freedom to publish is not adequate safeguard of independence when the freedom to propose is restricted.

We urge research institutions and individuals to take a long hard look at the way in which the motives of funding bodies

may subtly redirect and compromise research priorities and independence.

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## Imperial Japan

SIR—We should like to comment on the article (*Nature* 337, 107; 1989) in which Japan's deceased Emperor Hirohito was described as an admirable biologist. We fear that the article may be abused by those who wish to enhance or maintain the emperor system, and that it is likely to be cited in sentences such as 'A leading scientific journal, *Nature*, also admires the Emperor as a prominent biologist'.

Since Hirohito died on 7 January, programmes on television and articles in newspapers and magazines have been full of admiration and glorification for the personal qualities of the emperor. Neither the mass media nor leading intellectuals have touched on the dangerous and inadequate aspects of the emperor system, which have been used to control the ideology and attitude of Japanese citizens. Opinions against the emperor or the emperor system are suppressed by both direct and indirect pressure.

There are some problems in associating biology with the emperor. First, the words 'naturalist' or 'biologist' give a relatively good impression, and have been used to make an admirable personality of the emperor. Second, there are few faculty positions and departments in the fields of fundamental biology (except biotechnology and molecular biology) and funds are also scarce in Japan. But biologists can get money for international meetings, and other scientific activities in subjects related to those in which the emperor was interested. So some biologists have tended to use the emperor to get funds, while the government and conservative intellectuals have used such biologists and their activities to enhance the respectable image of the emperor. The International Biological Prize (established to celebrate the sixtieth year of the emperor's reign) is also used to enhance the image of Japan's emperor in the eyes of other countries.

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# Rise and fall of Western diseases

David J. P. Barker

Nutrition and hygiene during early childhood are important in determining the risk of the diseases which follow industrialization. We still know little about the processes involved.

THE term 'Western diseases' is used to describe a group of diseases common in industrialized countries but uncommon elsewhere<sup>1</sup>. These diseases are regarded as a consequence of the environmental changes accompanying industrialization and prosperity; it is often argued that their prevention depends on a return to practices of the past — for example, resumption of a diet high in complex carbohydrates and low in animal fats.

'Western' characterizes only the international distribution of diseases. It does not describe other features of disease distribution in populations. In particular, it does not predict changes in incidence other than the rise with the beginnings of industrialization.

Two main environmental changes during industrialization are in hygiene and in diet. Improvements in sanitation and housing lead to falls in mortality from infective diseases, especially among children<sup>2</sup>. Increased intake of total calories, animal fat, fruit and fresh vegetables, vitamins and minerals leads to better growth and development of children and contributes to the decline of infective diseases such as tuberculosis.

Epidemiological findings suggest that many diseases in industrialized countries fall into one of three groups, which can be shown by data from England and Wales, where numbers and causes of all deaths have been recorded for 140 years. The first group, associated with affluence, includes gallstones, renal stones and cancers of the breast, ovary and prostate. The incidence of these diseases has risen steeply during this century, particularly in more prosperous areas. The second group, associated with poor living standards, includes chronic bronchitis, stroke, stomach cancer and rheumatic heart disease. The incidence of these diseases has fallen during the past 50 years as living standards have improved. These diseases are commonest in the least affluent areas and in people with the lowest incomes. The third group is at different times associated with both affluence and poverty, and includes ischaemic heart disease, appendicitis and duodenal ulcer. The incidence of these diseases rose in the early part of this century, when they were more common among the rich. Subsequently the incidence fell and they are now commoner among poorer people.

The annual death rates from this last group of diseases in England and Wales<sup>3</sup>

are shown in Fig. 1. Together with those in the first group, they can be termed 'Western' diseases. Why have they declined when the environmental changes of industrialization have persisted?

Poliomyelitis was rare in Britain before this century but, as in other countries, it began to appear as hygienic and general living standards improved. This is in contrast to all the other common infective diseases, which were declining at this time. There was a sharp rise in notifications of poliomyelitis after the Second World War which persisted until the introduction of large-scale immunization (Fig. 1). It is now known that the rise of poliomyelitis resulted from the increasing vulnerability of the central nervous system to poliovirus infection with increasing age. As hygiene, sanitation and housing improved, the proportion of children escaping infection during the relatively safe period of infancy rose, and the number of cases of paralytic disease at later ages therefore rose in parallel.

The outbreaks of appendicitis which have accompanied industrialization in many parts of the world can similarly be explained as an age-dependent consequence of infection<sup>4</sup>. In England and Wales, death rates from appendicitis increased abruptly and steeply from around 1900, and then fell progressively from the 1930s onwards (Fig. 1). There is strong evidence that this trend reflects

changes in incidence of the disease, and similar trends were recorded in other European countries and in the United States. The explanation of these trends is thought to lie in the reduced levels of enteric infection in young children brought about by better hygiene, making them liable to develop appendicitis in response to infections at a later age. With continued improvements in hygiene, exposure to infection throughout childhood and early adult life became more uncommon. Because the appendix seems to be less vulnerable to infection after about 30 years of age, appendicitis declined.

Death rates from duodenal and stomach ulcers rose and fell in a similar way. The distribution of duodenal ulcer with social class was similar to appendicitis; while it was increasing it was more common among the rich, but as it declined it became commoner in poorer people. Recent findings of *Campylobacter*-like organisms in peptic ulcers suggest that the disease is spread by an infective agent<sup>5</sup>. The time trends point to age-dependent consequences of infection.

The trends in deaths from thyrotoxicosis (Fig. 1) show another process by which a rise in disease can be followed by a fall. Death rates rose to a peak in the 1930s and thereafter declined. Most deaths from this disease occur in the elderly, among whom toxic multinodular goitre is the usual cause of thyrotoxicosis. Analyses

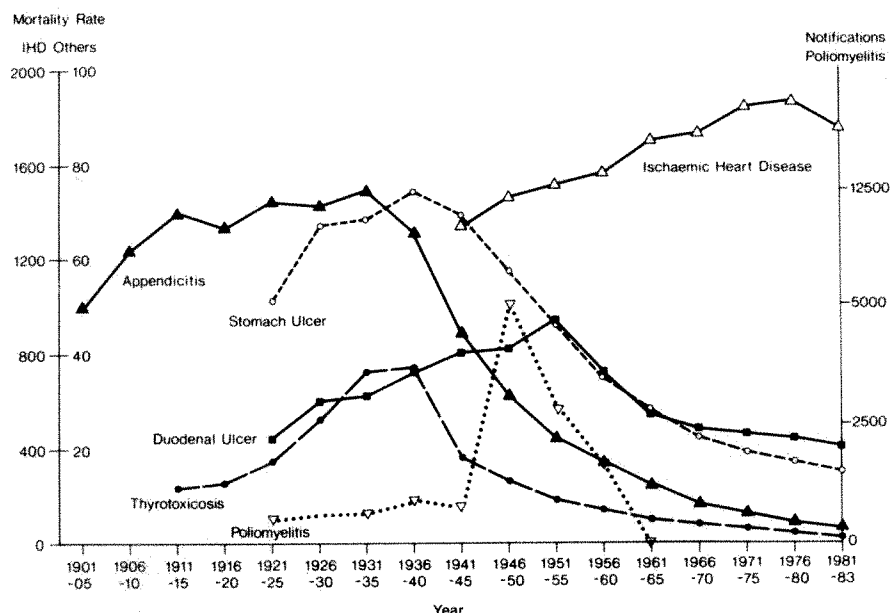


FIG. 1 Average annual mortality from selected diseases in England and Wales from 1901, and numbers of notifications of poliomyelitis in five-year periods<sup>3</sup>.

of age-specific rates in successive generations<sup>6</sup> show that rates rose progressively in people born after 1836 and reached a peak in those born between 1871 and 1886. This is shown in Fig. 2, which gives rates in each generation according to their year of birth.

Figure 2 also shows the progressive increase in dietary iodine in Britain during this century, as a consequence of diversification of diet and availability of iodine in many foods including fish, meat and milk<sup>7</sup>. Iodine deficiency during childhood was widespread among people born in Britain in the 1800s. Successive generations, however, were exposed to more iodine in adult life. There is evidence that people who are iodine deficient in youth are less able to adapt to increased iodine intake in later life and tend to develop thyrotoxicosis<sup>8</sup>. This would explain the rise in deaths from thyrotoxicosis in the early part of this century (Fig. 1). Successive generations born after 1880 were exposed to more iodine in childhood, which would have lessened their susceptibility to iodine in adult life; accordingly, thyrotoxicosis mortality fell from around 1940 (Fig. 1). This explanation of the time trends accounts for the apparent paradox that toxic nodular goitre is now common only in those areas of Britain where iodine deficiency used to be prevalent.

The essential process thought to underlie the trends of occurrence of toxic nodular goitre is a response to an environmental influence during early life, which has a critical effect on the ability to adapt to subsequent exposure. The same process may determine trends in ischaemic heart disease. Death from ischaemic heart disease was not distinguished from that from other forms of heart disease before 1940, but other evidence reveals a steep rise in rates during the first part of the century. From 1940, death rates continued to rise progressively and reached a plateau in the 1970s (Fig. 1). Since 1980 there has been a small decline. Similar patterns occurred in the United States, Canada, Australia and New Zealand; initial rises were followed by substantial falls, of around one-quarter in the past 30 years in the United States<sup>9</sup>.

Although death from ischaemic heart disease was more common in wealthier people during its steep increase in Britain, it is now more common in less affluent areas and among people with lower incomes. This geographical distribution is the reverse of that for other diseases thought to be caused by the Western diet — gallstones, renal stones and breast cancer are all more common in more prosperous areas. The geographical distribution of ischaemic heart disease is closely related to that of poor child health and development, indicated by high infant and child mortality about 70 years ago<sup>10</sup>. The aetiology of this disease may therefore

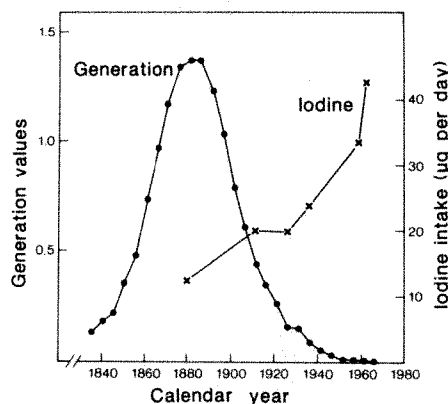


FIG. 2 Relative mortality from thyrotoxicosis in successive generations of women in England and Wales according to year of birth, together with estimated per capita daily iodine intake from milk, meat and fish<sup>7</sup>.

depend on two groups of environmental causes: one associated with affluence and likely to be mediated through a high-energy, high-fat diet; the other acting during childhood and associated with poor living standards. The rise in the disease results from an increase in the first, its fall from reduction in the second.

Further evidence of the effect of this second group of environmental causes is the inverse relation between risk of ischaemic heart disease and height, which is largely determined by growth in childhood. Long-term 'programming' of lipid metabolism during infancy, in response to infant feeding, is a mechanism for which there is increasing evidence in experimental animals and which could be a link between childhood and ischaemic heart disease. Another link may be the known inverse relation between fetal growth and blood pressure in adult life<sup>11</sup>.

Each of the diseases shown in Fig. 1 may rise and then fall in response to one environmental change that accompanies industrialization. Such diseases characterize the change from a mainly rural to a mainly industrial society. Responses to the childhood environment, including age-dependent consequences of infection and adaptation to diet during early life, may be important causative factors.

The search for causes of 'Western' diseases has concentrated on the adult environment. The importance of the childhood environment in determining responses throughout life may have been underestimated. Models of disease based on the effects of cigarette smoking, an influence in the adult environment which has been intensively studied, may have limited general application. Where differences in individuals' susceptibility to disease cannot be explained by differences in the adult environment, as is the case for ischaemic heart disease, they have often been attributed to genetic causes — especially if the disease has a familial tendency. Part of what is now regarded as the genetic contribution to ischaemic

heart disease may turn out to be the effect of the intra-uterine or early postnatal environment.

Critical adaptations during early life may determine optimal rates of change within populations. Appendicitis and duodenal ulcer became common at an early stage of improvements in hygiene. The size of epidemics of these diseases may depend on the speed with which hygiene improves throughout the population. The rise of appendicitis in Britain can be linked to the introduction of domestic hot-water systems. The introduction of piped water supplies was spread over more than half a century, piped water not reaching some rural areas until after the Second World War. Swifter execution of public-health reforms begun in the nineteenth century might have reduced the incidence of this disease.

By contrast, critical responses to nutrition in childhood, such as those that occur in toxic nodular goitre and that are suspected in ischaemic heart disease, may limit the extent of dietary change that a generation can be exposed to without adverse effects. It follows that improvements in living standards during early industrialization should be directed at children. Although the industrial revolution in Britain brought high wages to adults, children continued to grow up undernourished, in large families, and in poor, overcrowded homes.

Steep increases in the incidence of 'Western' diseases regularly follow industrialization in the developing world. Among people of Chinese origin, improvements in hygiene may occur without changes in the traditional diet. Elsewhere, as in the slums of many cities in the developing world, diet changes but poor hygiene persists. If more was known about the processes by which the environment in early life influences adult health, the hygienic and nutritional benefits which will accompany industrial development might be maximized, and the rise in incidence of 'Western' disease minimized. □

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# Heat conduction is a can of worms

Heat conduction is usually regarded as a phenomenon in which energy diffuses away from regions of high temperature, but diffusion may not always be a sufficient explanation.

WHETHER it is pleasing or otherwise to be told that a familiar principle is also a pack of lies is a matter of temperament. Some people are natural iconoclasts, others wish that at least some of the basic verities were eternal. But there are good reasons why what is known as Fourier's law (of heat conduction), which many people are still taught at high school, should be regarded as the crudest approximation to the truth. The interesting, but perplexing, question is that of what should take its place.

Most simply put, Fourier's law relates the flux of heat at any point in, say, a one-dimensional solid such as a metal bar to the temperature gradient at that same point. The heat flux and temperature gradients are taken as proportional to each other, with a constant of proportionality called thermal conductivity and with a minus sign (to allow that heat flows against the temperature gradient). The relationship is that of the diffusion equation, describing the diffusion of a chemical in a liquid.

High-school problem-solvers know how this can be used to calculate the change of temperature with time at any point in, say, a conducting bar. The rate of change with time of the temperature at any point, determined by the rate at which energy accumulates there, is evidently proportional to the rate of change with distance of the heat flux, with a constant which is the reciprocal of the specific heat and with another minus sign. The outcome is an equation relating the first time-derivative of the temperature and the second distance-derivative of the temperature. Given the distribution of temperature at the outset, it is then possible to calculate the temperature everywhere at all later times.

One might, for example, start with a metal bar at room temperature for half its length and at some higher temperature for the other half and follow the approach to equilibrium, when the temperature along the bar will be everywhere the same. But there is one obvious respect in which this result flies in the face of common sense: all solutions of these equations entail that the temperature everywhere along the bar will begin to change from the outset, however long the bar.

The implication is that the influence of the hotter half of the bar propagates instantaneously even to the most distant parts of the cooler half, which is unphysical. It is not merely that relativity disallows influences propagating faster than the speed of light, but that heat must get

from one end of the bar to the other by some physical process plainly overlooked by Fourier's law.

This is the starting point for a review earlier this year by D. D. Joseph of the University of Minnesota and Luigi Preziosi of the University of Naples (*Rev. Mod. Phys.* **61**, 41; 1989). Their objective is to define the circumstances in which heat can be propagated by means of waves, which has been observed in liquid helium and in some dielectric crystals at low temperatures. But their review inevitably begins with a discussion of the mechanisms of heat conduction and turns out to be a stimulating history of attempts to improve on Fourier's law, with the loose ends left dangling for all to see.

That there should be loose ends is not surprising; the mechanisms of heat transport are many and different. In a gas, energy is physically carried by molecules and transferred whenever they collide. In solids, on the other hand, lattice vibrations are a universal means of heat transport, but free electrons also play an important part in metals.

In all cases, the physical transport of heat from one place to another requires that some physical interaction should release energy at the destination — in a gas, the collision of an energetic molecule or, in a solid, the conversion of a quantum of energy in one vibrational mode into one or more quanta tied to other modes (which is possible only because of the anharmonicity of the vibrations of real crystals). Improvements on Fourier's law must take account of these processes, which involve microscopic energy conversion with characteristic times and distances (the time between collisions and the mean free path in a gas, for example).

Nobody will be surprised that James Clerk Maxwell is credited with the first important contribution to the argument, in 1867 (*Phil. Trans. Roy. Soc. Lond.* **157**, 49; 1867). Following logic and his instincts, Maxwell (working with his kinetic theory) concluded that the spatial gradient of temperature is not simply related to the heat flux, but to a combination of that and a quantity proportional to the time-rate of change of the heat flux. The constant of proportionality must evidently have the dimensions of time; numerically, it is a 'relaxation time' related (in Maxwell's case) to the interval between molecular collisions.

Joseph and Preziosi observe that Max-

well, having arrived at this conclusion, promptly discarded the extra term he had imported into the equation of Fourier's law on the grounds that the relaxation time must be negligible. In the event, his equation was rediscovered only in 1948, by C. Cattaneo. Mathematically, Fourier's law in its simple form boils down to a differential equation for the temperature (as a function of position and time) which is strictly equivalent to the diffusion equation (which is why the ratio of the thermal conductivity and the specific heat of a material is called its thermal diffusivity). Maxwell's formulation, and that of Cattaneo, are by contrast equivalent to the partial differential equation known as the 'telegraph equation', which is itself a modification of the more familiar wave equation and whose solutions are waves which are attenuated as they propagate. So why do we not more often encounter phenomena in which heat seems to propagate as waves, not by diffusion?

As early as 1917, Nernst was arguing intuitively that, at low temperature, propagating heat should have inertia of a kind; a pulse of internal energy arriving at some point in one direction might be expected to continue in the same direction in the absence of the influences that, at high temperature, would randomize the internal motions. That, said Nernst, should make wave propagation possible. In 1941, Landau developed the two-fluid theory of liquid helium II and predicted the existence of heat waves, recognizable as temperature oscillations, which he called 'second sound'. The prediction was confirmed in 1944, when Peshkov measured the speed of second sound in helium II. Peshkov also predicted wave-like heat propagation in low-temperature crystals, also now confirmed.

So why not room-temperature heat waves? Joseph and Preziosi take the view that the question cannot be decided until more is understood about the spread of relaxation times in the process of heat conduction. Meanwhile, they advocate a further modification of the equation corresponding to Fourier's law to take account of these refinements. Luckily, as their history of the field shows, all kinds of new techniques — molecular dynamics, for example — are now being brought to bear on the issue. And, for practical purposes, Maxwell's guess that the simple form of Fourier's law suffices for most purposes remains valid.

**John Maddox**



# All for one, one for all, that is our device

Jon Seger

SOCIAL insects have played a unique role in the development of the theory of natural selection, ever since Darwin noted<sup>1</sup> that sterile workers pose a "special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory". Darwin saw in outline how selection might be "applied to the family, as well as to the individual", an idea that eventually gave rise to the modern theory of inclusive fitness<sup>2</sup>. Inclusive fitness is a general concept that applies to all situations in which relatives interact, but the motivation to develop it (and in particular, its connections with sex-ratio theory<sup>3,4</sup>) came largely from social insects. Theories of group selection<sup>5</sup> have also been inspired by insect sociality, and may soon receive new impetus from the same source.

On page 420 of this issue<sup>6</sup>, Rissing and colleagues show that unrelated females of the desert leaf-cutting ant *Acromyrmex versicolor* seem to participate voluntarily in an unequal division of risk and labour during the establishment of new colonies.

Several different patterns of colony formation are found among social insects of the order Hymenoptera, and most have evolved more than once<sup>7</sup>. In some ants and bees, for example, a fraction of the parent colony's worker force departs with a newly produced female reproductive (gyne) who becomes the principal egg-layer (queen) of the resulting daughter colony. But in most social Hymenoptera, each gyne departs alone and attempts to establish a new nest and to raise a first

generation of workers without any help at all. In either case a colony has a single queen, and the relatedness of nestmates tends to be high.

The new colony may also be established by several or many gynes, without an initial worker force. This pattern is common among social wasps. Dominance hierarchies form among the cofoundresses, such that one or several high-ranking individuals monopolize reproduction, and subordinates act as workers. The average relatedness of nestmates varies widely, but cofoundresses seem to be significantly related in most species<sup>8</sup>.

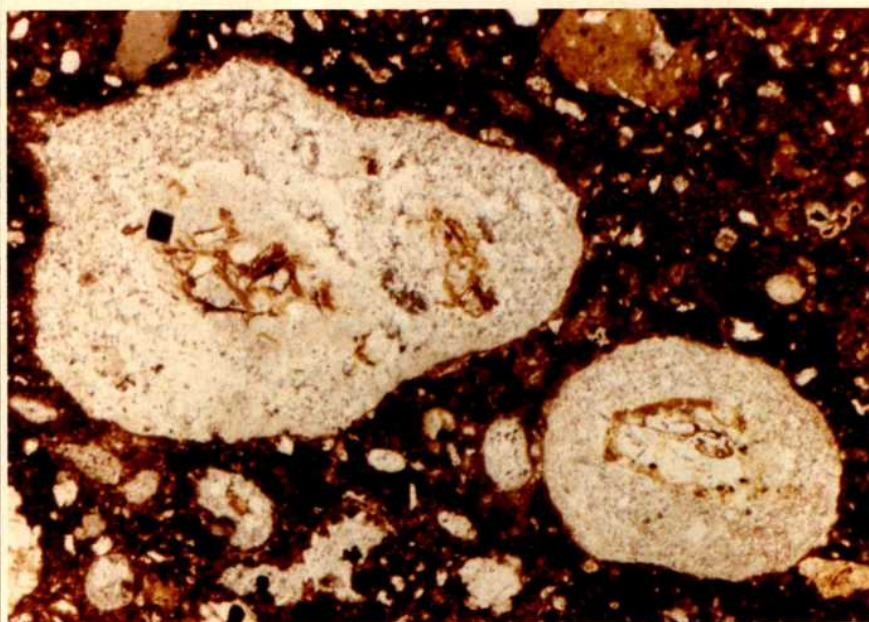
Multiple foundresses also occur in some species of ants; by analogy to social wasps, one would expect them to be relatives. In 1910, the great myrmecologist William Morton Wheeler<sup>9</sup> assumed "without doubt" that two cofounding females "were sisters that had accidentally met . . . and had renewed the friendly relations in which they had lived before taking their nuptial flight". Only in the past decade has it been learned that, in ants, cofoundresses are usually unrelated. The first evidence came from behavioural studies showing that gynes readily team up with each other independent of the distance between their natal colonies (and thus presumably independent of their relatedness)<sup>10,11</sup>. Recent genetic studies of relatedness within colonies of *Solenopsis invicta*<sup>12</sup>, *Veromessor pergandei*<sup>13</sup> and *A. versicolor*<sup>13</sup> show that cofoundresses are indeed no more closely related than random individuals drawn from the population at large.

In the laboratory, gynes have survived better in groups than alone<sup>10</sup>, and foundress groups of moderate size have produced more first-generation workers, more quickly, than have smaller or larger groups<sup>10,11</sup>. Species that frequently found nests cooperatively often engage in reciprocal brood raiding, with small colonies tending to be eliminated by their larger neighbours<sup>10,11</sup>. Thus at high population densities, cooperative founding may greatly increase the probability that a given nest will survive long enough to produce reproductives.

An alliance may improve survival, but given survival, it dilutes the expected reproductive success of each foundress. Conflict between foundresses or between workers and foundresses often begins soon after the emergence of the first workers<sup>10,11,14</sup>, and such conflict often leads to the expulsion or killing of one or more foundresses; in many species with multiple foundresses this process goes to completion and the mature colony is monogynous<sup>11</sup>.

In most ant species, foundresses raise the first workers on secretions derived metabolically from flight muscle and fat<sup>7</sup>, and they never leave the nest. *A. versicolor* is unusual in that one found-

## Life imitates (experimental) art



D. K. Bailey

THE white globules in this micrograph are chilled droplets of a carbonate melt, set in a groundmass of carbonate-rich volcanic ash. There has been much controversy as to whether such 'carbonatite' magma can form directly by partial melting of the mantle, or whether a two-stage process, involving modification of a mantle melt at shallower, crustal levels, is required. Recently, Margaret Wallace and David Green (*Nature* 335, 343–346; 1988; see also the accompanying News and Views article by J. Gittins) reported the experimental formation of a carbonatite melt by melting a mantle peridotite composition at mantle pressures and temperatures, lending support to the idea that carbonatites can be primary melts from the mantle. Now Ken Bailey on page 415 of this issue reports the existence of a close natural analogue to this experimental melt — the droplets pictured above, from the Rufunsa volcanoes in Zambia. Although these carbonatites have been known for some time, the similarity of their composition to that produced in the experiments, together with the discovery by Bailey of inclusions of mantle-type chromium-rich spinel (such as the black diamond-shaped grain in the larger droplet) now provides evidence that they are the long-searched-for primary carbonate melts. Field of view is 3.4 mm wide.

Laura Garwin



## TOPOLOGY

## Lowering the volume

Ian Stewart

ress forages outside the nest during the interval between nest establishment and the emergence of the first workers". Foraging is energetically costly and it exposes the forager to risk of predation. One might therefore expect the forager to be a subordinate foundress who was forced into the role by her dominant nestmates<sup>15</sup>, but Rissing and colleagues<sup>6</sup> find no evidence of conflict within foundress associations studied in the laboratory, either before or after emergence of the first workers.

Why should one foundress (the forager) perform such heroic service on behalf of nestmates to whom she is not related? The answer suggested by Rissing and colleagues is that efficiency matters above all else, in the race to establish a colony that survives the brood-raiding that will begin as soon as any neighbouring colony produces a worker force. Like her sedentary nestmates, the forager will have no chance to reproduce in the distant future if her colony succumbs early on. If the fates of cofoundresses are very tightly bound together then the appearance of selflessness may be only an illusion.

This argument seems to work best for the critical early history of the colony; it loses force as the colony matures and achieves greater security. When the time comes to produce reproductives, each queen would presumably wish the others gone. Rissing and colleagues observed 1.5 years of peaceful coexistence in their laboratory colonies<sup>6</sup>, which may well be a record for confounding ant species<sup>11</sup>. The story will become even more remarkable if this solidarity among unrelated queens remains unshaken throughout their lives. □

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ONE of the central aims of topology is to classify all possible manifolds up to topological equivalence. A manifold is a multi-dimensional analogue of a surface, and two manifolds are topologically equivalent if one can be deformed continuously into the other. The time-honoured method for classifying manifolds is to construct topological invariants: computable numerical or algebraic data that are the same for topologically equivalent manifolds but that distinguish between topologically inequivalent ones. For example, the number of holes in an ordinary two-dimensional surface is such an invariant. S.V. Matveev and A.T. Fomenko (*Usp. mat. Nauk.* **43**, 5–22; 1988; *Russ. math. Surv.* **43**, 3–24; 1988) have now made several fundamental advances concerning one of the most unusual and surprising invariants, the volume of a hyperbolic manifold.

The usual notion of volume is changed if the object concerned is stretched or bent, but this particular version of volume is extraordinarily 'rigid', and is not changed by continuous deformations. In addition, Matveev and Fomenko disprove a conjecture of Thurston (*Bull. Am. math. Soc.* **6**, 357–381; 1982) about the hyperbolic three-dimensional manifold (3-manifold) of minimal volume. Moreover, their methods have unexpected connections with fundamental questions in dynamics.

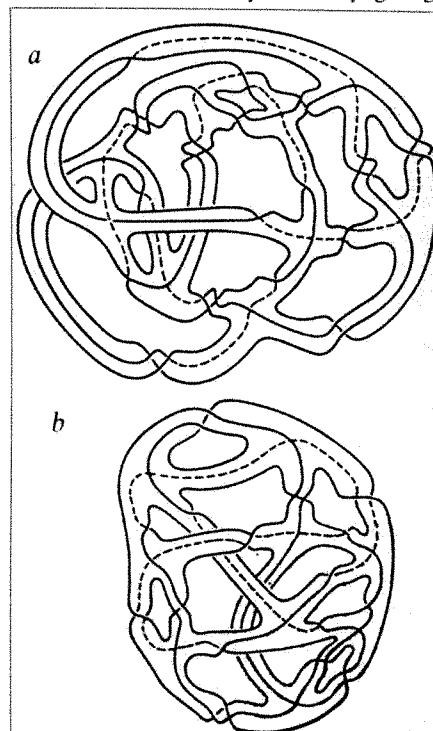
Over the past century, almost all of the objectives in the programme of finding invariants for manifolds have been accomplished for cases in which the dimension is either two, or greater than or equal to four. The remaining case, that of 3-manifolds, has proved far more subtle and intractable. The most encouraging new direction is embodied in recent conjectures of Thurston to the effect that every 3-manifold can be cut into pieces, each of which has a natural geometric structure (see my News and Views article in *Nature* **328**, 16; 1987).

This conjecture is at first quite startling, because geometry involves rigid concepts such as lengths and angles, which are destroyed by topological transformations. But the result has long been known to be true (and languished, unappreciated) in two dimensions. For two-dimensional surfaces there are three fundamentally different kinds of geometry: euclidean geometry; elliptic geometry, in which no parallel lines exist; and, in many ways the most interesting, hyperbolic geometry, in which there are infinitely many distinct lines through a given point and parallel to a given line.

Elliptic and hyperbolic geometry are the two possible types of non-euclidean

geometry in two dimensions. Elliptic geometry can be realized on the surface of a sphere, which has constant positive curvature; and hyperbolic geometry is valid on a surface of constant negative curvature. Euclidean geometry comes in between: it holds for flat spaces, of zero curvature.

A surface without boundary, such as a sphere or multi-holed torus, can be equipped with a unique geometric structure. For example, as already mentioned, the sphere carries an elliptic geometric structure. The standard torus, with one hole, can be obtained by abstractly 'gluing'



Combinatorial data defining *a*, Thurston's conjectured minimal-volume 3-manifold; and *b*, the new record holder identified by Matveev and Fomenko.

together the edges of a square. Because the square is contained in a flat plane, and no distortion of distances or angles occurs when its edges are 'glued', the torus carries the same euclidean geometric structure as its parent square. It turns out that all other surfaces, that is, tori with two or more holes, can be equipped with a hyperbolic structure.

Except in the euclidean case, the geometric structure has a natural length scale. Two spheres, for example, differ only in their radius. If the curvature (the reciprocal of the radius) is normalized to 1, then the radius must be 1. This fixes the length scale. The area must then be  $4\pi$ ; and this number, so determined, is a topological invariant. Any surface, if it can be

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### 100 years ago

#### THE GEOGRAPHICAL RESULTS OF MR. STANLEY'S EXPEDITION

It is evident from Mr. Stanley's stirring letters, which during the past week have cast all other topics into the shade, that pioneering in Africa is not yet at an end, and that the strange continent has not yielded up its vast wonder to knowledge.

Mr. Stanley's description of the character and extent of the Congo forest in his letter to Mr. Bruce is quite worth quoting:-

"Take a thick Scottish copse, dripping with rain; imagine this copse to be a mere undergrowth, nourished under the impenetrable shade of ancient trees, ranging from 100 to 180 feet high; briars and thorns abundant; lazy creeks meandering through the depths of the jungle, and sometimes a deep affluent of a great river. Imagine this forest

and jungle in all stages of decay and growth — old trees falling, leaning perilously over, fallen prostrate; ants and insects of all kinds, sizes, and colours murmuring around, monkeys and chimpanzees above, queer noises of birds and animals, crashes in the jungle as troops of elephants rush away; dwarfs with poisoned arrows securely hidden behind some buttress or in some dark recess; strong brown-bodied aborigines with terribly sharp spears, standing poised, still as dead stumps; rain pattering down on you every other day of the year; an impure atmosphere, with its dread consequences, fever and dysentery; gloom throughout the day, and darkness almost palpable throughout the night; and then, if you will imagine such a forest extending the entire distance from Plymouth to Peterhead, you will have a fair idea of some of the inconveniences endured by us from June 28 to December 5, 1887, and from June 1, 1888, to the present date, to continue again from the present date till about December 10, 1888, when I hope then to say a last farewell to the Congo forest."

From *Nature* 39, 560; 11 April 1889.

given a geometric structure of curvature 1, must have the same area  $4\pi$  as the sphere — and indeed in this case must be equivalent to a sphere. In a similar way negative curvature can be normalized to  $-1$ , and this also fixes a length scale. Therefore surfaces with a hyperbolic geometric structure also have uniquely defined areas.

For surfaces, far simpler invariants (such as the number of holes) achieve the desired classification, so this area has not featured prominently in most topologists' imaginations. But for 3-manifolds, analogously defined 'volumes' are turning out to be a much richer concept. As Thurston observed, there are eight distinct types of geometry in three dimensions. Three are euclidean, elliptic and hyperbolic geometry; the other five are harder to describe. Seven of these eight types of geometry are well understood, and so are the manifolds that carry such structures. The difficult case is that of hyperbolic manifolds: 3-manifolds that can be equipped with a geometry having constant negative curvature. For such manifolds one can normalize the curvature to  $-1$ , again obtaining a well-defined length scale; so the manifold has a uniquely determined volume. Moreover, this volume is a topological invariant — and a remarkably effective one.

Most invariants tend to be whole numbers. A simple example is the number of holes — clearly one cannot have half a hole. But the volume is a decimal. It is known that only a finite number of hyperbolic 3-manifolds have dimensions less than any given value. In particular there must be a smallest value. The corresponding manifold is presumably of some importance — otherwise why should it be the smallest one? So, which is it? Thurston conjectured that a particular manifold has

this smallest possible volume, and that its value is 0.98139.

Matveev and Fomenko use a new approach, combining two very distinct ideas: the complexity of a 3-manifold, and connections with dynamical systems. Complexity measures how complicated a combinatorial description of the manifold must be; the dynamical connection is described below. The complexity of a 3-manifold, which is a whole number, has many pleasant properties. For example, only finitely many manifolds have given complexity. The number of distinct 3-manifolds of complexity 0, 1, 2, 3, 4, 5 and 6 is precisely 3, 2, 4, 7, 14, 31 and 74, respectively. None of these is hyperbolic — in fact the first hyperbolic manifolds occur at complexity 9, and one of these is Thurston's conjectured manifold of minimal volume.

Using this notion of complexity, Matveev and Fomenko set up a computer algorithm to search for hyperbolic 3-manifolds and compute their volumes. They studied about a thousand manifolds in all. Among their list they found a new manifold, also of complexity 9, with the forbidding name  $(Q^2)_{8,2}$ , whose volume 0.94272 is smaller than that conjectured by Thurston. Indeed it is the only manifold in their list having smaller volume than Thurston's manifold, and they in turn conjecture that they have now found the true minimal volume. Because 3-manifolds involve 'curved space' they cannot be drawn directly, but their curious and intricate geometry may be gathered from two pictures (see the figure) showing the so-called singular graph for Thurston's manifold and the new, low-volume record holder. The singular graph determines certain combinatorial data from which the manifold can be constructed.

Matveev and Fomenko's very extensive

list of manifolds and volumes provides a wealth of valuable data against which to test conjectures. In particular their results cast doubt on one part of Thurston's programme for studying 3-manifolds. He suggests dissecting manifolds into simplexes (non-euclidean tetrahedra) and analysing how these fit together. But it now seems that some features of the mathematics depend on the manner in which this dissection is done, an unexpected difficulty.

Finally, there is a curious and deep connection with dynamics, which is central to Matveev and Fomenko's theory. Every hamiltonian dynamical system (that is, one in which there is no friction, so that energy is conserved) has associated with it a phase space determined by the position and velocity variables needed to specify its state. For example a mass moving in space requires three position coordinates and three velocity coordinates, and hence has a six-dimensional phase space. The subset of phase space corresponding to a fixed value of the energy is a manifold one dimension smaller — in this case 5.

For a simpler example, think of a simple harmonic oscillator (or idealized pendulum). This has one position coordinate and one velocity coordinate, giving a two-dimensional phase space. The constant-energy manifolds are concentric circles. But circles have positive curvature, hence elliptic geometry. Hyperbolic energy surfaces do not occur in two-dimensional phase spaces.

Matveev and Fomenko prove that something similar happens when there are two position coordinates and two velocity coordinates. The phase space then has dimension 4, and every constant-energy manifold has dimension 3. Thus a special class of 3-manifolds arises out of dynamics. Which? Under one further technical assumption, integrability, these manifolds can be characterized. They are precisely those 3-manifolds which can be cut into pieces, each having a geometric structure that is non-hyperbolic. Any of the other seven possible geometries may occur: hyperbolic geometry alone is forbidden.

Some of these ideas may sound outlandish, but they derive from long-standing themes in mainstream mathematics. What is new is the manner in which they have been brought together. Volume, a rigid geometric concept, now applies in a topological context; it is related to complexity; and hamiltonian dynamics somehow conspires to forbid even a tiny part of a constant-energy surface having constant negative curvature. The story is a remarkable and important affirmation of the power of the new geometric approaches to topology and dynamical systems. □

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# Glycine maintains excitement

Alan C. Foster and John A. Kemp

THE explosion of interest in the NMDA (N-methyl-D-aspartate) receptor, one receptor subtype for the excitatory neurotransmitter L-glutamate, has generated several new concepts of transmitter function. Thus, it is now known that NMDA-receptor activation is conditional on a unique voltage-dependent regulation of channel conductance by magnesium<sup>1,2</sup>, and that the neuronal response consists of both short-term membrane conductance and long-term biochemical changes, the

concentrations of glycine increase the frequency of NMDA-receptor channel opening in a strychnine-insensitive manner, and suggested a mechanism involving allosteric regulation of the receptor complex through a distinct glycine binding site. This idea is supported by the existence of strychnine-insensitive <sup>3</sup>H-glycine-binding sites which have an anatomical distribution identical to that of the NMDA receptor<sup>3</sup>. It seems likely that the glycine-binding site is an integral

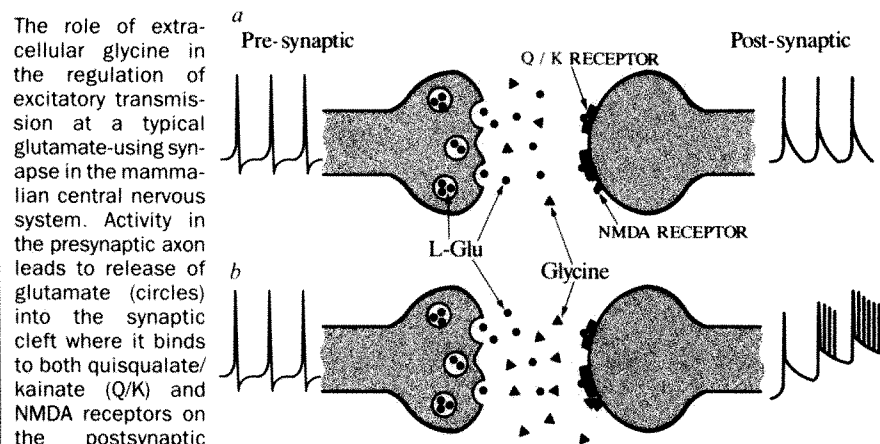
the onset of desensitization, but accelerates the recovery of the receptor from its desensitized state.

This is the first time such a mechanism for the regulation of a ligand-gated ion channel has been proposed; it may explain the large enhancement of NMDA responses achieved by glycine. If this were the sole mechanism through which glycine exerts its effects, then the co-agonist idea would be negated. But patch-clamp studies with the selective glycine antagonist 7-chlorokynureate, using a fast perfusion system similar to that of Mayer *et al.*, indicate that this compound can produce a complete block of NMDA responses<sup>11</sup>. Thus, glycine binding could have two effects, one involved with the initiation of NMDA-receptor activation and the other to speed up recovery from desensitization.

Many questions surround the role of glycine in the regulation of NMDA receptors *in vivo*. NMDA responses obtained in slices of adult central nervous system *in vitro* are generally not enhanced by glycine (or its analogue D-serine), but can be blocked in a glycine-reversible manner by glycine antagonists such as kynureate, 7-chlorokynureate and HA-966<sup>11-13</sup>. Thus, the facilitation of NMDA responses by glycine occurs in adult neurons, but under these experimental conditions extracellular glycine levels are sufficiently high to occupy all of the glycine-binding sites.

It has been suggested<sup>4</sup> that this situation may be the case *in vivo*, as levels of glycine in the cerebrospinal fluid are high (greater than one micromolar). Thompson *et al.*<sup>6</sup> report that glycine enhances NMDA responses in slices of cortex when applied in close proximity to the neuron under study in conditions of reduced perfusion, which are claimed to mimic more closely the situation *in vivo*. This agrees with recent studies *in vivo* which have shown a facilitation of NMDA responses by locally applied glycine or D-serine<sup>14-16</sup>.

Thompson *et al.* also demonstrate a



latter caused by elevated intracellular calcium concentrations<sup>1</sup>. Furthermore, receptor activation involves not only the binding of an agonist, such as L-glutamate or NMDA, but also an allosteric facilitation by glycine binding to a separate site within the receptor complex<sup>1</sup>. Two papers in this issue provide further insights into this effect of glycine. On page 425, Mayer *et al.*<sup>5</sup> present evidence that at least part of the enhancement of NMDA responses by glycine occurs through a previously unknown mechanism — an acceleration of recovery from desensitization. And on page 422, Thompson *et al.*<sup>6</sup> demonstrate that glycine facilitates NMDA receptor-mediated neuronal excitation in slices of cerebral cortex, suggesting that glycine is involved in the tonic regulation of transmission.

The effects of glycine on the NMDA receptor are distinct from its long-established role as an inhibitory neurotransmitter in lower brain areas and spinal cord, mediated by a strychnine-sensitive chloride conductance. Johnson and Ascher<sup>7</sup> have shown that submicromolar

part of the receptor complex, because glycine facilitation is maintained in isolated membrane patches<sup>1</sup> following solubilization of NMDA receptors<sup>8</sup>, and in NMDA receptors expressed in *Xenopus* oocytes following injection of rat brain messenger RNA<sup>9</sup>. The effects of glycine on the NMDA receptor have been compared with those of benzodiazepines on the GABA<sub>A</sub> receptor, but the magnitude of the enhancement of NMDA responses by glycine is much greater than that seen with benzodiazepines on GABA responses. Indeed, Kleckner and Dingledine<sup>10</sup> claim that no NMDA-receptor response can be obtained in the absence of glycine, and propose that receptor activation requires the binding of both glycine and L-glutamate as 'co-agonists'.

The new findings of Mayer *et al.*<sup>5</sup> are of direct relevance to this problem and add a new facet to the glycine story. They suggest that the main effect of glycine is to prevent desensitization, a decline in the receptor response which occurs during a prolonged agonist application of the NMDA receptor. Glycine does not block

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facilitation of synaptic responses by glycine. This was observed only in the fraction of synaptic responses which exhibited an NMDA receptor-mediated component under these experimental conditions. This effect of glycine is likely to be a widespread phenomenon, as similar observations have been made in hippocampal neurons in culture<sup>17</sup> and in thalamic neurons *in vivo*<sup>18</sup>; and can be inferred at various synapses in the central nervous system from earlier studies using HA-966<sup>18,19</sup> which is now known to be a selective glycine antagonist<sup>13</sup>. Thus, glycine facilitation of synaptic responses mediated by NMDA receptors may be a common regulatory mechanism at excitatory synapses.

Clearly, it is now of interest to define the processes that regulate extracellular glycine concentrations in regions of the brain where NMDA receptors play important roles. Alterations of these systems would lead to profound changes in

neuronal activity mediated by NMDA receptors (see figure). The possibility that endogenous glycine antagonists (such as kynurenate) may be involved in regulating neuronal function should also be considered. If the main facilitatory effect of glycine is to accelerate recovery from desensitization of the NMDA receptor, it is likely that glycine will act in physiological situations where prolonged responses are required and repeated activation is necessary, such as induction of long-term potentiation. Conversely, a pathological excess of glycine could lead to prolonged excitation and epileptiform activity, contributing to the overactivation of NMDA receptors suspected to occur in seizure disorders and neurodegenerative disease. □

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## EXTREME ULTRAVIOLET ASTRONOMY

# A new window on the Universe

*P. Gondhalekar and C. Jordan*

THERE are few spectral ranges in which observations of astrophysical sources have not been made. One of the few is the extreme ultraviolet region, which is virtually unexplored owing to earlier assumptions that the interstellar medium would be opaque at wavelengths between the hydrogen Lyman photoionization edge (91.2 nm) and about 30 nm. But during the past decade there has been increasing evidence that the absorbing interstellar material is not uniformly distributed. The growing optimism concerning the possibility of detecting this unexplored radiation from various sources was reflected at a recent meeting\*. It became clear that observations in the extreme ultraviolet could provide crucial tests of current theories concerning objects as diverse as active galactic nuclei, cool dwarf stars and the jovian aurora. The growing confidence in, and enthusiasm for, the new astronomy was amply demonstrated by the presentation of many plans for suitable telescopes for future space missions.

The turning point for extreme-ultraviolet astronomy came in 1975 with the detection of emission from the white dwarf HZ43, by an instrument on the Apollo-Soyuz mission. More recently, several nearby hot white dwarfs were observed with the Voyager ultraviolet spectrometers (J.B. Holberg, University of Arizona). Soft X-ray spectra of some sources, including cool stars in the proximity of the Sun, were obtained with the X-ray satellite, EXOSAT (N.E. White,

ESTEC) which was sensitive to wavelengths up to about 40 nm.

Also, the case for observing Solar System objects in the extreme ultraviolet has already been established by spectra of the giant planets and their satellites, obtained from Voyager 1 and 2. For example, strong lines of oxygen and sulphur ions are observed from the Io plasma torus. Improved spectra extending to wavelengths below 60 nm should allow an improved understanding of the plasma processes causing the jovian auroral emission as ions precipitate from the magnetosphere (P.D. Feldman, Johns Hopkins University; W.M. Harris and J.T. Clarke, University of Michigan; S. Chakrabarti, University of California, Berkeley).

The local interstellar medium is of great interest because the distribution and density of the local gas determines the direction and distance to which extreme ultraviolet sources can be detected. The most accurate neutral-hydrogen column densities come from measurements of ultraviolet absorption in spectra of discrete stellar sources, but these do not give sufficient spatial coverage to establish the global distribution. All-sky surveys at 21 cm, the principal hydrogen radiowave emission line, do give full coverage but the spatial resolution is too coarse to measure the hydrogen opacity in a particular direction. (C. Heiles, University of California, Berkeley; D. York and P. Frisch, University of Chicago).

There is a diffuse, hot (10<sup>6</sup> K) component of the interstellar medium which is an important source for ionizing helium, but

its location and how pervasive it is are questions still much debated (F. Bruhweiler and K.-P. Cheng, Catholic University; P. Jackobsen, ESA). The overall picture that is emerging is that the local region is relatively highly ionized and thus has low opacity, rather than being devoid of material; there are higher neutral-hydrogen mass column densities at larger distances in the galactic plane, particularly towards the Galactic Centre. Large molecular-cloud complexes, such as those in Taurus and Auriga, add regions of high absorption but there are galactic longitudes (around 230°) where absorption is lower than average.

It seems unlikely that any small 'windows' exist through which extra-galactic sources might be viewed at the longer wavelengths (Heiles), but there is a good chance that active galactic nuclei and quasars could be observed up to wavelengths of 20 nm (H. Marshall, University of California, Berkeley). The general advice for observing to great distances is to observe out of the galactic plane, as the colder, more dense medium has a smaller scale height. There is sufficient uncertainty in the distribution of the hot and cold components however, to guarantee that extreme-ultraviolet surveys will add considerably to our knowledge and understanding of the interstellar medium.

There is little doubt that the local interstellar medium will be transparent to extreme-ultraviolet radiation from many types of stars. Existing observations of X-ray emission from main-sequence B (hot, blue-white) stars lead to models which predict the presence of two component, wind-driven shocks with temperatures in the range 10<sup>5</sup>–10<sup>7</sup> K. New spectra could further test these theories (J. Cassinelli, University of California, Berkeley). The solar extreme-ultraviolet spectrum, which gives a good indication of what might be expected from cool main-sequence stars, has been extensively observed at high spectral and spatial resolution, and many spectroscopic diagnostic techniques for measuring electron densities and temperatures have been tested (G.A. Doschek, US Naval Research Laboratory).

Similar spectra of nearby cool stars would allow emission lines formed in the temperature range 2 × 10<sup>4</sup>–10<sup>6</sup> K to be observed, leading to improved models of the structure and energy balance in stellar coronae (C. J.). At present, only average temperatures and densities can be found for stellar coronae, using broad-band X-ray measurements. Spectra and observations of the temporal variation of lines formed at different temperatures would allow more precise determination of coronal conditions and of the contribution made by stellar active regions. It is important to improve measurements of coronal densities and temperatures in order to

\*Berkeley Colloquium on Extreme Ultraviolet Astronomy, Berkeley, California, 19-20 January 1989.



understand known correlations between coronal X-ray fluxes, chromospheric line fluxes and stellar rotation rates. These must be controlled ultimately by the non-thermal heating and the energy loss processes. A wide range of more exotic stellar objects, including symbiotic stars, AM Herculis binary stars, and cataclysmic variables, should also provide exciting

new data (A.K. Dupree, S.J. Kenyon and J.C. Raymond, Harvard-Smithsonian Center for Astrophysics; D.Q. Lamb, University of Chicago). □

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## A marriage of convenience or necessity?

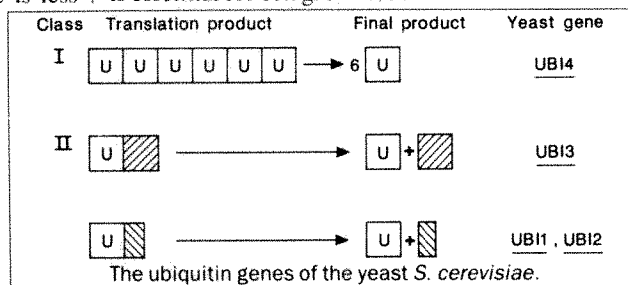
Jonathan R. Warner

UBIQUITIN, a 76-amino-acid protein, is, as its name implies, ubiquitous in eukaryotic cells (see ref. 1 for a review). A complex enzymatic system attaches it to proteins, usually by peptide bond formation with the  $\epsilon$ -amino of a lysine, either singly or in branched multiple ubiquitin structures. Ubiquitin can be a signal for the protein-degradation system of cells, much as a blaze on a tree serves as a signal for the logger. But in others its embrace is less lethal; it is reversibly attached to the histone H2A without causing its degradation<sup>2</sup>. Its function here is unknown.

Ubiquitin is remarkably conserved through evolution, with a similarity of 96 per cent between yeast<sup>3</sup> and human<sup>4</sup>, and between yeast and *Trypanosoma cruzi*<sup>5</sup>. Even more remarkable is the conservation in the arrangement of the genes encoding ubiquitin, which are found in two basic forms in most organisms (see figure). Class I is a polyubiquitin gene which encodes a polypeptide of up to 100 uninterrupted, tandemly repeated ubiquitins presumably released by proteolysis at the Gly-Met peptide bond that joins the repeats. Class II is a fusion between a single ubiquitin and one of two other sequences, of either 52 or 76–80 predominantly basic amino acids, again remarkably conserved through evolution. In this issue these basic sequences are identified, in the yeast *Saccharomyces cerevisiae* by Finley *et al.*<sup>6</sup> on page 394, and in mammals by Redman and Rechsteiner<sup>7</sup> on page 438, as members of another common group, ribosomal proteins. Finley *et al.*<sup>6</sup> fused antigenic tags to the *UBI1* and the *UBI3* gene products of yeast and find the 52-amino-acid *UBI1* tail in the 60S ribosomal subunits and the 76-amino-acid *UBI3* tail in the 40S subunits. A protein with the amino-terminal sequence of the *UBI3* extension had previously been identified as ribosomal protein S37 (ref. 8). By immunoblotting with antibodies made against a peptide within the mammalian 80-amino-acid extension, Redman *et al.*<sup>7</sup> find the same protein in the 40S subunits

and identify it as mammalian ribosomal protein S27a. Although less conserved through evolution than ubiquitin, yeast S37 and mammalian S27a are similar. Protein sequence data indicate that the ribosomal proteins from both organisms have been cleaved from the ubiquitin moiety without the loss of any amino acids.

By deletion analysis, Finley *et al.*<sup>6</sup> show that the 52-amino-acid ribosomal protein is essential for cell growth, as are all other



eukaryotic ribosomal proteins they tested. It is therefore surprising that cells without the gene encoding ribosomal protein S37 do grow, although poorly. These cells have a severe deficiency of 40S ribosomal subunits, apparently because of a defect in the final processing step(s) in the formation of 18S ribosomal RNA. Does the lack of S37 influence only ribosome assembly, or does it also have some effect on the rate or accuracy of translation?

It is a remarkable finding that the sole source of two ribosomal proteins is derived from ubiquitin fusion genes. But it is truly extraordinary that this arrangement has persisted throughout eukaryotic evolution, even into the freakish trypanosomes. Is ubiquitin dependent on ribosomal proteins or vice versa? Or is there a mutual symbiosis?

Introns are rare in the genes of *S. cerevisiae*, whereas most of the genes encoding ribosomal proteins have a single intron. Although it is not yet clear what selective pressure has maintained introns in the ribosomal protein genes, it is interesting that the members of the duplicate pair, *UBI1* and *UBI2*, each have an intron in the ubiquitin-coding portion of the gene.

Another conserved region of ubiquitin

genes is the heat-shock promoter element<sup>3,5</sup>. In several organisms, including yeast, the polyubiquitin genes are responsive to stress, but the fusion genes are constitutively expressed. In the case of the yeast genes, this is presumably because of the ribosomal protein enhancer elements, UAS<sub>rp</sub>, found upstream of the fusion genes<sup>3</sup> and almost all ribosomal protein genes<sup>10</sup>. The fusion genes provide the main source of ubiquitin during log growth, when ribosome synthesis is active. The polyubiquitin gene, *UBI4*, is derepressed only late in the growth cycle, during nitrogen starvation or after heat shock — all conditions under which ribosomal protein synthesis is repressed. Ubiquitin need not be derived from the fusion genes, however. If ubiquitin sequences are deleted from all the fusion genes, the cell still grows simply by turning on the polyubiquitin gene<sup>8</sup>.

The ubiquitin moiety of *UBI3* is helpful but not essential in the function of ribosomal protein S37. If S37 is expressed from a *UBI3* gene from which the ubiquitin-coding sequences have been deleted, many copies of the mutant gene are needed to replace fully one copy of the *UBI3* gene. One could speculate that the ubiquitin is used as a translational spacer for a very short protein, as a nuclear localization signal or as a 'wedge' to assist in assembling proteins into the ribosome. At what point in the assembly process is the ubiquitin cleaved? An alternative view derives from the observation that the ribosomal proteins turn over very rapidly if not assembled into a ribosome<sup>11,12</sup>. Ubiquitin could protect the ribosomal proteins from degradation, or perhaps cleavage from a ubiquitin molecule is a stratagem to make a protein without an amino-terminal methionine.

Although neither ubiquitin nor ribosomal protein seems essential for the other, there must be some explanation for this ancient and enduring marriage. That explanation may reveal the original role of ubiquitin, or some hitherto unsuspected connection between protein synthesis and protein degradation. □

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# Origins of marginal basins

Leg 124 shipboard scientific party\*

THE Sulu and Celebes seas are tectonically critical, but poorly known, marginal basins in the western Pacific Ocean. On Leg 124, completed in early January, we sought to determine the age, stratigraphy, palaeo-oceanography and current state of stress in the basaltic basement of these basins, drilling at five sites (see figure). We discovered unusually fine magnetostratigraphy in the basins, including the documentation of a magnetic reversal event within the Matuyama chron (approximately 1.1 million years (Myr) ago), which has been observed in few locations previously.

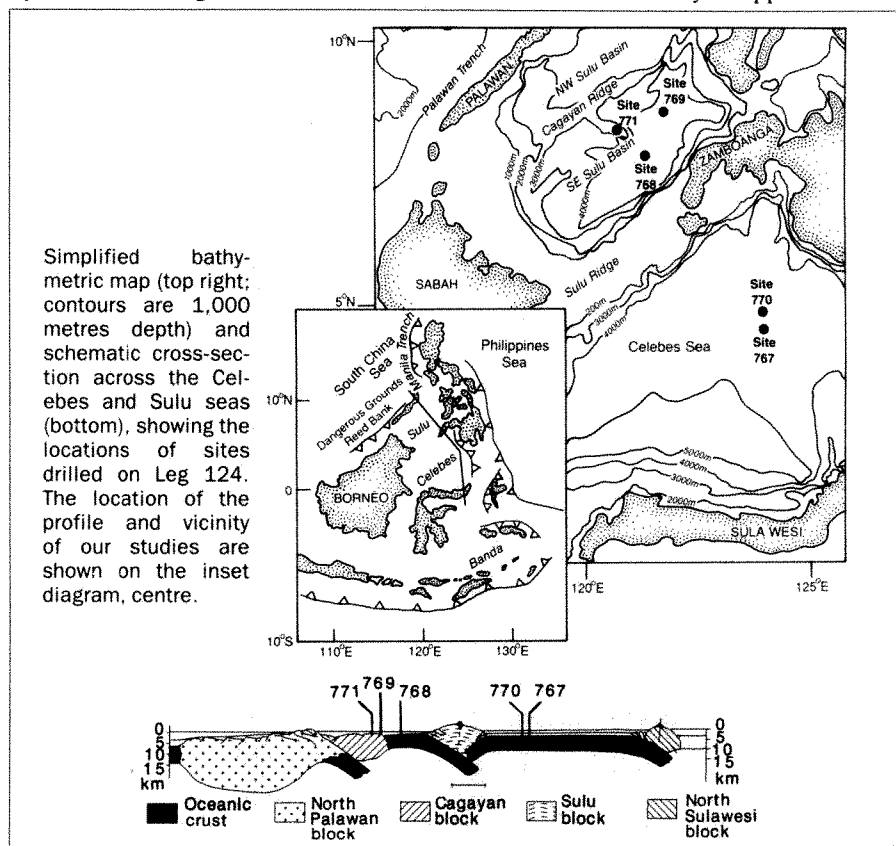
The western Pacific region is characterized by a series of marginal basins bordered by festoons of island arcs. The Sulu, Celebes and Banda basins are thought by some to represent an originally continuous, trapped fragment of a once-larger ocean basin, and by others to have formed by back-arc spreading. Our results rule out a single origin for all the basins, and it seems most likely that the origins of the Celebes and Sulu basins are very different. The basins lie in a broad zone of collisional tectonics, and one of our principal objectives was to use the sedimentary record of the basins to constrain the history of collisional events. Also, stress orientations within the basins establish the primary forces active in this complex zone of collisional tectonics, and the comparison of these results with those from Leg 123 in the south-east Indian Ocean should be instructive.

Basement underlying the Celebes Sea is plagioclase-olivine phyric basalt, and the Sulu Sea basement is composed of olivine basalt. We recovered pillow basalts at both sites in the Celebes Sea — 100 m at site 770 but only 42 cm at site 767. The cores from site 770 include sill and glass-rimmed pillow basalts and intercalated massive, veined flows. We drilled to a depth of 222 m at site 768 in the Sulu Sea basement, recovering a series of pillow basalts and sheet flows, intruded by

two dolerite sills. The trace-element geochemistry of the Celebes Sea basement (site 767) bears the signature of normal mid-ocean ridge basalts (MORB), whereas the Sulu Sea basalts seem to be transitional between MORB and island-arc tholeiites.

The stratigraphies of sediments in the basins yield a detailed record of their histories. The Celebes Sea originated in an open ocean setting in the middle Eocene

as a back-arc or intra-arc basin in the late early to early middle Miocene as determined by radiolarian biostratigraphy in sediments overlying basement. This age coincides with the collision of the Cagayan Ridge with the rifted continental margin of China. Initial deposition of volcanic brown clay was followed shortly thereafter by coarse tuffs from rapidly deposited pyroclastic flows. Slow accumulation rates ( $9 \text{ m Myr}^{-1}$ ) marked the early part of the middle Miocene, but in the late middle Miocene (10.5 Myr ago), very rapidly deposited ( $300 \text{ m Myr}^{-1}$ ) continentally derived turbidites inundated the basin. These volcanic ash layers appeared in the



(42 Myr ago). This age is constrained by the biostratigraphy of clays bearing radiolarians (planktonic protozoa) directly above basement. This determination is consistent with previously determined magnetic anomalies in the basin (Weissel, J.K. *Am. Geophys. Un. Geophys. Monogr.* 23, 37–47; 1980). By the middle Miocene (14 Myr ago) the sea was receiving continentally derived turbidite deposits rich in quartz and plant debris. By the late Miocene (6 Myr ago) nearby explosive arc volcanism was producing thin ash layers in the basin, and continued to provide a major component to the basin sediments until recently. No major carbonate units occur at site 767, but thin carbonate turbidites were deposited sporadically in the middle to late Miocene and more commonly in the Pliocene (5.1–1.6 Myr ago) at this site.

The Sulu Sea seems to have originated

basin 6 Myr ago, indicating the initiation of some of the volcanoes that presumably surround the basin.

A significant change in sediment type from green clay below to nannofossil marl above occurred 1.9 Myr ago, probably indicating the time of rapid shallowing of the surrounding sills, isolating the basin from deep waters of the Pacific. This was also a period of rapid shallowing of the calcite compensation depth worldwide, which could also have contributed to some of the change in sedimentation in the Sulu Sea.

Sites 769 and 771 on the flank of the Cagayan volcanic ridge, penetrated a section similar to that at site 768 in the Sulu Sea, but devoid of turbidites, and therefore very much thinner (280 and 240 m of sediments, respectively). Brown claystone at the base of the section at site 769 contains late early to early middle

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Miocene radiolarians, and marl at site 771 contains radiolarians and nannofossils of this age, the same as that of the Sulu Sea and the cessation of volcanism on Cagayan Ridge. We also found volcanic tuffs at site 769 beneath the brown claystone, but these tuffs are basaltic to andesitic in composition, indicating a different source from the rhyolitic to dacitic tuff in the deep Sulu Sea.

Logging at sites 770 and 768 included use of the borehole viewer, which measures the shape and roughness of the hole in the basement. The holes tend to be elliptical, with their long axes lying perpendicular to the direction of maximum horizontal stress. In places the long axes tend to develop small 'breakouts', which are easily visible in the record. The method thus gives a measure of stress direction. Preliminary results at both sites indicate that the maximum horizontal stress direction trends to the north-east, which is consistent with the expected stresses related to the Miocene and Pliocene collisions of the Sulu, Cagayan and Palawan ridges with the Philippine mobile belt.

#### PALAEONTOLOGY

## Sea-dragons all aswim

Michael A. Taylor

THREE major finds of fossil marine reptile on display in a new exhibition\* in Bristol will fuel the current revolution in marine reptile studies. The existence of several different kinds of large predator in the same sea poses difficult questions about their ecological partitioning. Two of the new finds are ichthyosaurs from the Lower Liassic (about 200 million years ago) and the third is the skull of a pliosaur, from the rather younger Kimmeridge Clay of Westbury, Wiltshire, UK (see figure).

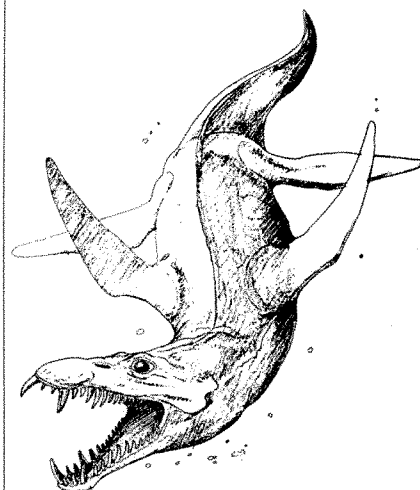
A virtually complete skeleton of an ichthyosaur from Charmouth<sup>1</sup> in Dorset, UK was about 9 m long in life. Although comparable in length with other large Liassic ichthyosaurs, its long, delicate snout and tiny, sharp teeth contrast with their robust snouts and heavier teeth. The large size of the Charmouth ichthyosaur combined with a narrow snout suggests a diet of small, abundant prey near the base of the food chain. But marine reptiles never seem to have exploited filter-feeding on even smaller prey, as do the modern mysticete whales, filter-feeding sharks and the newly reinterpreted giant teleost fish *Leedsichthys*<sup>2</sup> from the Jurassic Period.

The only known specimen of the much smaller ichthyosaur *Excalibosaurus costini*<sup>3</sup> is also on display in Bristol. This

A short, normally polarized, full-reversal event within the Matuyama chron is evident in the palaeomagnetic record. Occurring just below the Jaramillo sub-chron 1.1 Myr ago, this reversal corresponds to the Cobb Mountain event documented by E.A. Mankinen *et al.* (*Geology* **6**, 653–656; 1978) and is supported by marine magnetic-anomaly profiles described by D.K. Rea and R.J. Blakely (*Nature* **255**, 126–128; 1975).

Maturity of the terrestrial type of organic matter at Site 768 reaches the stage of thermal hydrocarbon generation at a shallow sub-bottom depth, indicating a thermal gradient greater than 100 °C km<sup>-1</sup>. We even observed some thermogenic generation of gas at this site.

Calcium and magnesium in interstitial waters show positively correlated increases with depth in the lower parts of the sedimentary sections of sites 767–770. Because normal alteration products of basalt scavenge magnesium, these observations suggest the presence of a chemical mechanism of crustal alteration previously unsuspected in the oceanic crust. □



Restoration of the pliosaur *Liopleurodon macromerus*, a 7.5-m-long oceanic predator of 150 million years ago (courtesy of John G. Martin and Bristol City Museum and Art Gallery).

of fast teleost fish has been implicated in the marine reptiles' decline: many had disappeared from the seas long before the end of the Cretaceous period (65 million years ago).

The exhibition celebrates Bristol Museum's acquisition and curation of these three major finds. But it also narrates the museum's key role in the history of research on marine reptiles, and laments the destruction, in an air raid in 1940, of the original collection of marine reptiles, the finest in Britain outside London<sup>12,13</sup>. It was here during the 1820s that W. D. Conybeare and H. T. De la Beche carried out the first accurate assessments of ichthyosaurs and plesiosaurs, interpreting them as links in the "great chain of being" between fish and reptiles<sup>14</sup>. These marine reptiles helped to popularize palaeontology, and — ironically — to give the impetus leading to the replacement of the paradigm of the great chain by darwinian evolution. Now the cycle is complete and the marine reptiles are once again receiving well-deserved attention. □

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animal, from Somerset, is distinguished by a long, protruding upper jaw, presumably for slashing through schools of prey too small to catch individually.

The Westbury pliosaur is a fortunate exception to the destruction of fossils by modern quarry machinery before they are even spotted<sup>4</sup>. The 1.6-m-long skull of *Liopleurodon macromerus* is exceptionally well preserved, and comes from an animal perhaps 6 to 7 m long. The robust jaws, strong pointed teeth with cutting keels, and wide gape indicate a predator which killed and tore apart any prey which it could catch<sup>5,6</sup>.

The ecological diversity of ancient seas must have been very great to have supported all these large, carnivorous sea-dragons simultaneously. The next task is to sort out the adaptations of each in order to gain a better appreciation of their ecological niches. One approach is to use tooth form and wear, supplemented by stomach contents, to assess dietary specialization<sup>6</sup>. Another is to compare locomotor adaptations<sup>7–10</sup>: the fish-shaped ichthyosaurs and the compact, short-necked plesiosaurs were probably fast pursuit predators, while the longer-necked plesiosaurs may have used their long necks to ambush smaller prey while cruising. Despite the presumed speed of ichthyosaurs and plesiosaurs, they were probably slow when compared with modern forms such as toothed whales<sup>9–11</sup>. The evolution

\*The Great Sea Dragons, City of Bristol Museum and Art Gallery, Queen's Road, Bristol BS8 1RL, 17 February–6 May 1989.

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# Painting by resonance

Chris Scarre

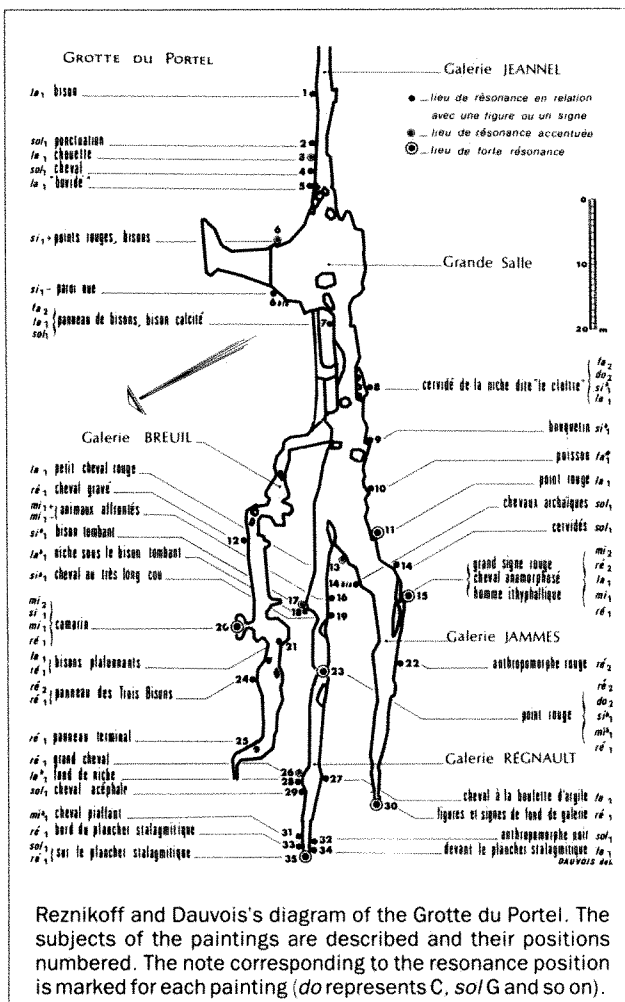
DID the painted caves of western Europe once resound to the music of Palaeolithic chants? Such is the thesis put forward by Iégor Reznikoff and Michel Dauvois in the latest issue of the *Bulletin de la Société Préhistorique Française* (85, 238-246; 1988). The authors have studied three caves in the Ariège department at the foot of the French Pyrenees. Their results suggest that the acoustics of the caves played a significant part in determining where the paintings were located, and this observation leads directly to the supposition that music or chants were important elements in cave ceremonies around 20,000 years ago.

Reznikoff and Dauvois rely on the fact that in certain places ("points of resonance") the caves resonate in response to particular notes. They proceeded slowly through the cave using their voices to produce a series of notes spanning almost three octaves, from C<sub>1</sub> to G<sub>3</sub>. They extended the range of notes for a further two octaves by harmonics and whistling. Where there was a resonance response, they recorded the location and the particular note eliciting the response. They used these observations to draw up a resonance map of the cave (see figure).

The resonance of the caves is not in itself surprising, but the significance of the study becomes apparent when the authors compare their points of resonance with the location of cave paintings. They draw three main conclusions. First, most of the cave paintings are at or within 1 metre of points of resonance. The Grande Salle at Portel, for example, which gave no resonance response, also has relatively few paintings. Second, most of the points of resonance correspond to locations with cave paintings. Indeed, the best points of resonance are always marked in this way. Finally, the authors claim that the location of some of the paintings can be explained only by the resonance of that particular location. A good example is number 23 at Portel (see figure), where a particularly effective

point of resonance is marked by red painted dots, as there is not enough room for a full painted figure.

Reznikoff and Dauvois remark from their own experience on the impressive effect of cave resonance, which would have been all the more striking in the flickering half-light of the simple lamps or tapers used by the original artists. Drums, flutes and whistles may have been used in cave rituals — bone flutes have been found at several Palaeolithic sites in Europe of roughly the same age as the paintings. The potential of cave resonance



would, however, be elicited only by the much greater range of the human voice. The image of the cave artists chanting incantations in front of their paintings may not be too fanciful. Reconstructing prehistoric sounds is inevitably a risky and ambitious venture, but this study is of particular value in drawing new attention to the likely importance of music and singing in the rituals of our early ancestors. □

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# Time out of mind

LIKE many busy people, Daedalus often wishes there were more hours in the day. Indeed, he has given serious thought to slowing down the rotation of the Earth, but still cannot think of a suitable dumping-ground for the surplus angular momentum. Instead, he is exploring the possibility of speeding up the human internal clock. In most people its free-running period is about 25 hours, entrained with more or less reluctance to the 24-hour day by the pitiless light of morning. But those infuriating eager-beavers who snap into action at the crack of dawn presumably have a cycle of less than 24 hours, and so adjust to the natural day with some phase-lead.

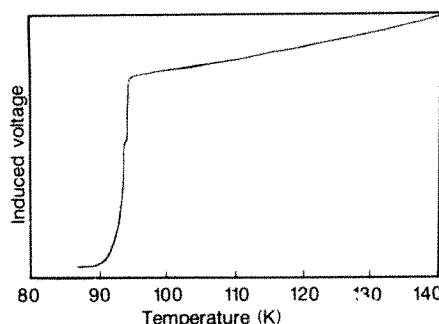
Many other creatures also have an inbuilt circadian rhythm. It seems to be genetically determined; hamsters and fruitflies sometimes occur in mutated forms with a different circadian period, or even with none at all. These mutations seem to work by altering the output of some time-regulator protein. If the same mechanism works in man, then the human internal clock could be neatly adjusted by altering the output of this protein. So DREADCO's pharmacologists are trying to develop a drug which either blocks the receptors for the timing protein, or inhibits or facilitates its production. In the current state of knowledge, this has to be a hit-and-miss business; but the hormone melatonin and the benzodiazepine tranquilizers, both of which affect the biological clock, seem a good place to start. Serum analysis of volunteer sluggards and human dynamos may also be revealing.

DREADCO's Regulator® tablets will bring wonderful harmony to all our lives. Those who (like Daedalus) find that morning comes too soon, will shorten their cycle by cautious doses of the appropriate Regulator until they are in perfect tune with the clock. Their short-cycle brethren will lengthen their subjective day to make the most of the evening. But Daedalus is most intrigued by the possibility of a special Regulator which, like the *per<sup>0</sup>* mutation in fruitflies, does not merely alter the circadian rhythm but actually abolishes it. Acyclic individuals will live a very unusual life. They will show no particular tendency to go to sleep; once asleep, however, they will show no particular tendency to wake up. Having no time-sense, they will never be bored; and they will only eat when actually hungry. Acyclic workers will take over all the continuous industrial processes which are currently manned imperfectly, and often dangerously, in shifts. They will severely test theories about the importance of sleep and dreams to human mental functioning. And if our lifespan is indeed counted off in periods of the circadian rhythm, they should live forever.

David Jones

# Effect of N<sub>2</sub> on superconductors

SIR—Matthews *et al.*<sup>1</sup> reported that the superconducting transition temperature ( $T_c$ ) of YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7</sub> ( $y \approx 7$ ) ceramic can be increased by exposure to cold gas. Exposure to nitrogen raised  $T_c$  by nearly 40 K. Sarma *et al.*<sup>2</sup>, however, performed experiments which led them to suggest that this observation could be explained by condensation of nitrogen gas in the porous ceramic, but Matthews and colleagues have supported their own interpretation by further experiment<sup>3,4</sup>. It is clearly important to establish whether or not the transition temperature of a superconducting ceramic can indeed be raised by such a simple procedure. In an independent investigation, we find no effect of cold nitrogen gas on  $T_c$ .



Superconducting transition of encapsulated and unencapsulated half-disks of YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7</sub> ceramic.

Our samples were disks of YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7</sub> ceramic, about 10 mm in diameter and 2 mm thick, supplied by N. Alford of ICI Advanced Materials. Superconductivity was detected by measurements of a.c. susceptibility. The temperature of the specimen-holder was varied by means of the metal-cylinder apparatus described elsewhere<sup>5</sup>, in which the specimen holder is held in a vertical column of cold nitrogen gas along which a temperature gradient is established. The temperature of the specimen holder can be varied by raising it to the appropriate height, and was measured using a silicon diode thermometer. When measuring  $T_c$ , the temperature was varied at less than 1 K min<sup>-1</sup>, a rate for which preliminary experiments showed a negligible temperature difference between the specimen and the thermometer. The induction coils were separated from the specimen by a few millimetres so that the surfaces of the latter were exposed to the cold nitrogen gas.

The changes in  $T_c$  reported in refs 1, 3 and 4 are so large (up to 40 K) that they should be easy to observe. Nevertheless, to ensure that any change would be detected, we cut one of the specimen disks in half along a diameter. One half was encapsulated, by means of a chemical-dip treatment, and the other was left untreated. The two halves were placed together

between the coils. If the nitrogen gas does indeed alter  $T_c$ , we should observe two transitions, one in the encapsulated half at the original transition temperature and one in the unencapsulated half at a higher temperature. The a.c. susceptibility of a pair of specimens, as they were first cooled from room temperature to 80 K, is shown in the figure. The small step in the voltage drop shows that the two halves have very slightly different transition temperatures ( $\sim 0.3$  K) and that our apparatus would detect any change in the  $T_c$  of the unencapsulated specimen.

Experiments were performed on six samples, four of 15% porosity and two of less than 2% porosity. Specimens were exposed to nitrogen gas at 80 K for up to 43 hours, the transition temperature being measured at intervals of a few hours. No change in  $T_c$  was observed for any specimen; the variation in a.c. susceptibility with temperature after exposure to cold nitrogen gas was no different to that observed during the initial cooling.

Thus we have not been able to reproduce the increase in  $T_c$  observed in refs 1, 3 and 4, nor indeed did we detect any change at all in  $T_c$  resulting from exposure to cold nitrogen gas. Moreover, although many other experiments must have involved exposure of ceramics to cold gas for long periods, there is, as far as we are aware, no report of a resulting increase in  $T_c$ . We cannot explain the results of Matthews, Taylor and co-workers; there may be a flaw in their experimental method, possibly of the kind suggested by Sarma *et al.*<sup>2</sup>, or perhaps their samples are not typical of YBaCuO ceramics.

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## Antigen receptor tail clue

SIR—I wish to draw attention to an amino-acid motif present in the cytoplasmic tails of several protein components of the antigen receptors on T cells and B cells<sup>1,2</sup>, as well as the immunoglobulin E (IgE) receptor (FcRI) on mast cells<sup>3</sup>. These receptors show similar structural and functional properties; they consist of several different transmembrane proteins which upon cross-linking generate a signal resulting in cell activation.

As shown in the figure, the motif is found in the CD3- $\gamma$ , - $\delta$  and - $\zeta$  components of the T-cell receptor (but not the CD3- $\epsilon$  or TcR- $\alpha$  or - $\beta$ , components); in the MB1 component of the immunoglobulin M antigen receptor on B cells and on the FcRI- $\beta$  and - $\gamma$  components of the receptor for IgE on mast cells (but not the FcRI- $\alpha$  component). When the con-

sensus sequence motif derived from these components was used to search the NBRF protein databank for related protein, two were identified: a B-cell-specific membrane protein, B29, of unknown function<sup>4</sup> and the envelope protein, gp30, of bovine leukaemia virus (BLV)<sup>5,6</sup>.

The first two specified amino acids of the motif are the negatively charged aspartic acid (D) or glutamic acid (E); the third and fifth are tyrosine (Y); and the fourth and sixth are leucine (L) or isoleucine (I). All six are located with a precise spacing between each other, and would lie on the same side of an  $\alpha$ -helical barrel if the cytoplasmic sequence formed an  $\alpha$ -helix. On cross-linking of receptor, the conserved amino acids from several chains may come together to form a binding site for the putative proteins that generate the

h CD3- $\gamma$	GQDGVRSRASDKQTLLPNDOLYQPLKDKREDDOYSHLOGNQLRRN
m CD3- $\gamma$	-----Q-E-----Y-----KK
h CD3- $\delta$	-HETG-L-G-A-T-A--R--V--R--D-A--G--WA-N-
m CD3- $\delta$	-HETG-P-G-AEV-A--K-E--R--T--S-G--WP-N-KS
m CD3- $\zeta$	ADAYS DIGTKGERRRGKGH-G--G-STATK-T-DA-HMQT-APR
BLV gp30	LKLLRQAPHFPEISLTPKP-SD-A-LPSAPEI--SPVKPDYINLRPCP
h MB-1	RKRWQNEKLG-AGDEYEDEN-EG-NLDDCSM-EDISRGLOGTYQDVGSLNIAD
m MB-1	RKRWQNEKFGV-MPDDYEDEN-EG-NLDDCSM-EDISRGLOGTYQDVGNLHIGD
m B29	DKD-GKAGMEE-HT-EG-NIDQTAT-EDIVTLRTGEVKWSVGEHPGQ
r FcRI- $\gamma$	RLKI-V-KA-IASREKS-AV-TG-NT-NQET-ET-KHEKPPQ
r FcRI- $\beta$	Y-IGQEF-E-RSKV-D-R-EE--HVYSPI-A-EDTREASAPVVS
Consensus:	DxxxxxxDxxYxxLxxxxxxYxxL E E I

Sequences are aligned by means of the six consensus amino acids of the motif (boxed). Identical amino acids are indicated by -, gaps by :; h, human; m, mouse; r, rat. For all sequences except CD3- $\zeta$  and BLV gp30, the sequence shown begins with the first cytoplasmic amino acid; for all except MB-1 (whose human sequences our unpublished sequence) and B29, the sequence shown ends with the C terminus of the protein.

signals resulting in proliferation and differentiation of the B and T cells, and histamine release from the mast cells. A similar signal might be generated by the viral protein gp30 explaining the polyclonal B-cell proliferation (persistent lymphocytosis) connected with BLV infection.

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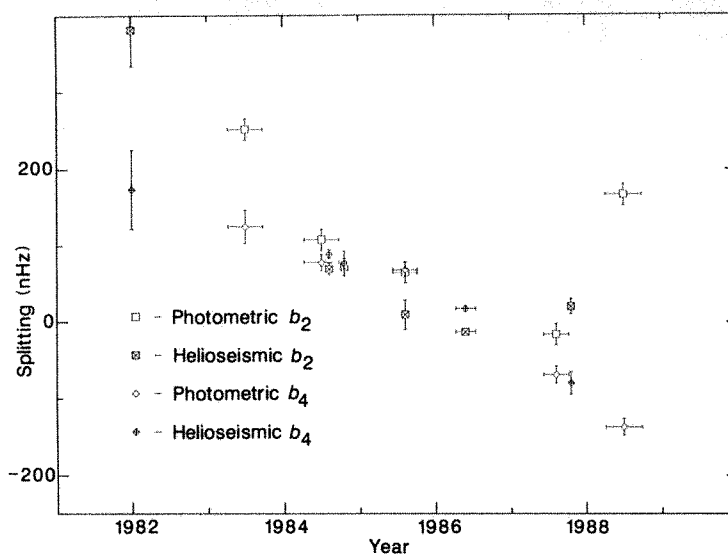
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## Solar oscillation

SIR—Gough discusses the recent helioseismic evidence for solar-cycle variations in the global solar aspherical structure. But he does not mention that the variable, even, helioseismic-splitting coefficients; the solar-limb temperature measurements; and the solar-constant data together yield a consistent interpretation of solar asphericity. Gough speculates that the splitting coefficients are directly related to sunspot number, and using a Legendre polynomial decomposition of the surface distribution of sunspots, makes a prediction<sup>1</sup>. It is not obvious, however, that the sunspot number (surface density) and frequency splittings should be plotted on the same graph. This approach contrasts with my earlier calculation<sup>2,3</sup> which explicitly predicts the splittings from an asphericity in the sound speed. These model predictions have been confirmed by observation<sup>4</sup>.

In his report<sup>1</sup>, Gough criticizes the limb temperature observations and inferences because they do not account for the variability of the mean solar irradiance measured by ACRIM. In fact the limb data nicely explain<sup>5</sup> the observed cycle variation of the solar constant. Furthermore, the formalism and data directly provide information on the depth dependence of the asphericity. For example, I have used the helioseismic observations to show that the asphericity is best described by a perturbation in the outer superadiabatic layer of the convection zone<sup>6</sup>. Thus, unlike Gough, I find it hard to see how the helioseismic data can be interpreted to explain a measureable solar-cycle variation in the neutrino flux from the Sun's core.

In any case, Gough's 'prediction' may soon be tested. Splitting data from 1988 should show large changes (and significant centroid frequency shifts) from 1987. In contrast with Gough<sup>1</sup> I expect the  $b_1$  ( $\alpha_1$  in ref. 2) splitting coefficient to be even



Time dependence of the helioseismic splitting measurements<sup>3,4</sup>. The most recent photometric observations are from limb photometry obtained in 1988. In contrast with Gough<sup>2</sup>, I expect the  $b_4$  coefficient to decrease in 1988.

smaller than in 1987 (see figure). Note that this is not a correlative inference, but a test of a simple model consistent with observed solar irradiance variations.

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GOUGH REPLIES — That the even splitting coefficients of solar 5-minute oscillations should arise predominantly from a latitudinal variation in the propagation speed  $c$  of acoustic waves rather than a geometrical distortion of level surfaces is a natural supposition. The frequency splitting  $\delta\nu/\nu$  between sectoral and zonal modes was  $\sim 2 \times 10^{-4}$  in 1982 near the solar maximum<sup>7</sup>, whereas the oblateness at that time was probably no greater than  $2 \times 10^{-5}$  (ref. 8). Moreover the frequency splitting induced by the oblateness is of opposite sign to that observed.

What is the nature of the variation in  $c$ ? The most natural thought is that it is a sound-speed variation, associated with a variation in temperature. Simple models of the solar envelope in which thermal asphericity is induced by magnetic inhibition of convection in the equatorial regions, yet which maintain radial hydrostatic balance, reproduce the splitting data<sup>9</sup>. But they require a photospheric temperature variation that is a hundred times greater than recent observations indicate<sup>6</sup>. What is remarkable, however, is Kuhn's finding<sup>3,4</sup> that if the observed latitudinal variation in relative temperature<sup>6</sup> away from the poles is assumed to extend essentially unmodified through the superadiabatic convective boundary layer, then the splitting data are reproduced.

It was the original failure of plausible hydrostatic-envelope models to explain the splitting data that induced an investigation of magnetic effects, particularly as the asphericity was similar to the sunspot

distribution. A non-uniformly distributed fibril magnetic field of magnitude similar to that observed reproduces the required frequency splitting<sup>9</sup>. It was that result that led me previously to assume a correspondence between magnetic activity, measured by sunspot density, and  $\delta c/c$ .

From an extrapolation of the sunspot data from 1 January 1988 I thus predicted the results of measurements being carried out in Antarctica by a team from the US National Solar Observatory. Recently, R. K. Ulrich kindly provided me with Mt Wilson sunspot data up to 25 November 1988. Using the scaling from my letter<sup>1</sup> these data provide a revision of the prediction to  $\alpha_1 \approx 170$  nHz,  $\alpha_2 \approx -110$  nHz; thus in contrast with my previous extrapolated result  $\alpha_1$  seems still to be declining, in agreement with Kuhn's expectations.

The new discussion of limb brightness measurements<sup>6</sup> was not available when I wrote my first report<sup>1</sup>. The impressive correspondence with the ACRIM data lends considerable credence to the interpretation in terms of effective-temperature variations, and thus demands a consistent theoretical investigation of its implications concerning the dynamical balance of the convection zone and the splitting of oscillation frequencies.

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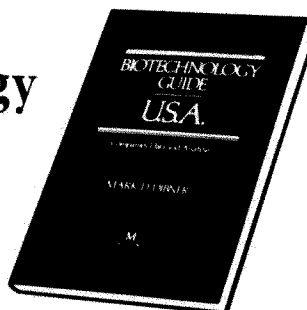
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# A way with words

Stephen Jay Gould

**The Oxford English Dictionary, 2nd edn.** Prepared by J.A. Simpson and E.S.C. Weiner. Clarendon: 1989. Twenty volumes, £1,500, \$2,500.

THE Brothers Grimm, after a lifetime of service collecting folk tales and developing some basic principles of linguistic evolution, began their greatest project in 1838 — a German dictionary that would illustrate the history of all words found in three centuries of literature, from Luther to Goethe. As the architects of mediaeval cathedrals understood so well, some projects are too big to complete in a lifetime, and satisfaction must reside in a worthy start and a workable plan. Wilhelm, who died in 1859, only reached the letter D, while Jacob, who lived four years longer, got all the way to F. Their great work was finished in our own century.

When James A. H. Murray became the first editor of *The Oxford English Dictionary* in 1879, he faced an even more daunting task — for his project would document all English words in all meanings, active and extinct, from 1150 up to the moment, and including actual quotations for all nuances of use. (The starting point was arbitrary, but sensible as a criterion for the inception of English, allowing about a century after the conquest of 1066 for the Romance language of the victorious Normans to fuse sufficiently with the indigenous Germanic of the vanquished Anglo-Saxons.) Murray anticipated a labour of ten years, and a product of four volumes and 6,400 pages. In 1884, with half his time elapsed, Murray published the first volume, reaching halfway through the first letter to 'ant'. But, as Aesop's grasshopper discovered, industry is an ant's middle name. Murray and his worldwide network, ranging from full-time editors to volunteers from Madras to California, persisted. The task was finished in 1928, long after Murray's death.

A project such as the *OED* cannot end so long as the genealogical skein of English-speaking people persists — for language is the hallmark of humanity, and linguistic change is the primary record of history. Any stated completion could only be an admission of failure. At most, the guardians of this greatest monument in the history of English scholarship may choose, now and again, to mark a way-

station in their eternal updating by issuing a hard-copy version of their dynamic storehouse. (Such overt summations may become less and less necessary as computer technology permits continuous change in electronic versions, with immediate access to all revisions and additions as they occur; though a few die-hards who love the heft, feel and smell of a real volume may — God preserve them forever — keep the art of bookmaking alive.) This second edition, in 20 volumes of 21,728 pages, defining about half a million words with illustrations from about 2.4

million quotations, is both a progress report and one of the greatest causes for celebration in the history of publishing.

The *OED* is timeless in the ethical sense of possessing ultimate and permanent value *in se*. But it is also the child of its time in form and purpose. It began as a project of the Philological Society in 1857 (an effort that delivered nearly 20 years of preliminary work to Murray's later editorship — and he still took five years to reach ant!). Two years later, in 1859, the Society published guidelines for the great work, including two fundamental principles: that "it should contain every word occurring in the literature of the language"; and that "in the treatment of individual words the historical principle will be uniformly adopted". This crucial decision, the foundation of the *OED* ever since, reflects the most enduring and innovative theme of nineteenth-century scholarship in general — the use of historical connection and development as the chief principle of organization and explanation. Geology had burst the bonds of time and provided an ample stage; biology had discovered the genealogical basis of all natural order; anthropology and linguistics were reconceived as historical sciences. It is no accident that these guidelines for the *OED* were published in the same year as Darwin's *Origin of Species*.

In the *OED*, all words are treated as historical entities with origins in etymologies and histories of changing usage. The earliest meanings are presented first, whatever their current status, followed by later developments in genealogical sequence. Since words evolve in the manner of organisms, the editors faced the same problem confronting darwinian taxonomists who had to translate the actual geometry of evolutionary branching into the linear format of a book. They adopted the biologist's convention of listing primary meanings ('species') in linear order (by arabic numerals in the *OED*), but gathering them by groups into higher taxa ('genera'), each representing a separate branch (roman numerals in the *OED*).

This format frees the *OED* from a dictionary's conventional role as the arbiter of 'correct' usage. Standard modern definitions have no pride of place or special designation; they take their proper order in the historical flow. But this format also makes the *OED* the greatest reference book ever written — for it turns each word into a story, and its totality becomes our history, if not our very definition as human. In a world of contingency, no essence exists beyond the labyrinthine and

**ant** (ænt). Forms: 1 (*W. Sax.*) æmete, -ette, -ytte, 3-4 amete (amote), amte, 4-6 ampte, 5-6 ante, 5-7 annt, 6- ant. Also 1 (*Anglian*) \*ēmete, 3-4 emete (-atte), 4-6 emote, 6 emmette, -otte (-ont), amyte, emet, 6-7 emmot(t, 6- emmet. *Pl. ants* (1 æmetan, 2-4 ameten, 4 amptes). [OE. æmete, ēmete, cogn. w. OHG. āmeiza, WGer. \*āmaitjō, f. ā- (see *Æ-* pref.) off, away + maitan, ON. meita, OHG. meizan 'to cut,' as if 'the cutter or biter off.' (Graff.) The OE. became in 12-13th c. āmete or ēmete in different dialects; āmete has by suppression of medial vowel and bringing together of two consonants become amte (ampte), ante (cf. account for accompte), ant; ēmete, retaining the medial vowel, is now EMMET, q.v. *Ant* is the leading literary form.]

1. a. A small social insect of the Hymenopterous order, celebrated for its industry; an emmet, a pismire. There are several genera and many species, exhibiting in their various habits and economy some of the most remarkable phenomena of the insect world. (For other quotations see EMMET.)

c. 1000 Sax. Leechd. 1. 87 æmettan ægru genim. 1297 R. GLOUC. 296 As pycke as ameten crepep in an amete hulle. 1340 Ayenb. 141 Alsuo ase pe litel amote. 1382 WYCLIF Prov. xxx. 25 Amptis [1388 amtis] a feble puple, that greithen in rep time mete to them. 1430 LYDG. Chron. Troy 1. i. He sawe by the earthe lowe Of Antes crepe passing greate plente. 1533 ELYOT Cast. Helth iii. xii. 66b. The lyttelle ant or emote helpeth up his felowe. 1547 BOORDE Brev. Health clxi. 58 Amytes, or Pysmars, or Antes. 1585 LLOYD Treas. Health Bviii. Powder of Amptes, myxte with Oyle. 1578 MASCALL Planting (1592) 58 For to destroy Emets or Antes, which be about a Tree. 1611 BIBLE Prov. vi. 6 Goe to the Ant [Wycl. ampte, amte, Coverd. Emmet], thou sluggard. 1633 G. HERBERT Ch. Mil. in Temple 184 The smallest ant or atome knows thy power. 1642 SIR T. BROWNE Relig. Med. 30 The wisdom of Bees, Annts and Spiders. 1733 POPE Ess. Man iii. 184 The Ant's republic, and the realm of Bees. 1838 Penny Cycl. X. 372 Formic Acid, or acid of ants. 1861 HULME Moquin-Tandon II. iv. 1. 213 When the Red Ant (*Formica Rufa*) crawls over a piece of litmus paper, it produces a red track.

b. In colloq. phr. to have ants in one's pants (orig. U.S.), to fidget constantly, esp. because of extreme agitation, excitement, nervousness, etc.; to be impatient or restless. Cf. ANTSY a.

[1939 KAUFMAN & HART *Man who came to Dinner* 1. ii. 62 'I'm in love.' 'I'll pull you out of this Miss Stardust. I'll get the ants out of those moonlit pants.'] 1940 'C.



unpredictable paths of historical development. The *OED* is the ultimate companion for those lonely years on a desert island in our standard *Gedanken* experiment. We consult it for a definition, and invariably emerge with an adventure. Half a million stories to last for as many thousand-and-one nights as any person can endure. (I'm not sure that I would trade my *OED* even for Scheherazade herself.)

Start anywhere. 'Chemistry' began with two extinct meanings as alchemy and paracelsian versus galenic medicine. Its modern definition dates from the seventeenth century, but the vernacular meaning of "instinctual and unanalysable attraction or affinity between people" is even older — dating from a comment of Queen Elizabeth (the first one) in 1600, and not to the beaches of modern California. 'Physics', thanks to Aristotle, is an even older word, but it encompassed all nature (even including God and spirits according to Locke) until eighteenth-century usage began to split the organic and inorganic worlds. 'Biology' is a relative newcomer, dating only from the German Treveranus and the Frenchman Lamarck in 1802 (with the first use in English in 1813).

'Science' (from the Latin *scire*, to know) goes back to the beginning, but as a general word for systematized knowledge of any sort (Chaucer wrote about "a science of good works"). The modern usage follows a series of branching and progressive confinement. Science began to separate from art in the eighteenth century, but restriction to knowledge about the physical and natural world only occurred in the mid-nineteenth century. Keeping abreast of the latest developments, the final definition presents 'science park', with an American source of 1972 for first usage.

Related words teach us more. 'Scientism' was used in a purely descriptive sense as early as 1877, but the modern derogatory meaning dates from G.B. Shaw in 1921 — in *Back to Methuselah*, and with a capital S ("The iconography and hagiology of Scientism are as copious as they are mostly squalid"). The concept of 'scientist' may date from the Domesday Book, but the word only goes back to 1834. The *Quarterly Review* then noted "the want of any name by which we can designate the students of the knowledge of the material world collectively". "This difficulty was felt very oppressively" at a recent meeting

of the British Association for the Advancement of Science. The nameless attendees considered philosopher as "too wide and lofty" and "savans" (*sic*) as "rather assuming". "Some ingenious gentleman" then suggested "scientist" by analogy with artist, claiming "no scruple" for the odd termination of *ist* by noting



James A.H. Murray, word collector extraordinary.

its acceptance in "economist" and "atheist". "But this was not generally palatable", the *Quarterly* reports, and scientist might have died aborning, if William Whewell had not taken up the cause in 1840. And a good thing too — or we might have been saddled with "scientman", proposed once in 1636, but quickly exterminated.

The three-page entry on 'nature' is a fascinating treatise on English thought about essential qualities (the original definition of properties held by birth, or natively). Category I speaks of the "essential qualities of things"; II of vital or physical powers in humans, including two extinct meanings ("semen or menses" in a lineage extending from Chaucer to James Joyce, and "the female pudendum, especially that of a mare" extending from Caxton through to the eighteenth century); III of the power or impulse directing or controlling human action (as opposed to grace); IV (now getting close to modern scientific usage) as regulative powers that control the physical world, or

even (but not until the seventeenth century), "the material world with its objects and phenomena". This last (and the basis, I assume, for the name of this journal), is category IV, definition 13 in the *OED*. Nature, by the way, has been personified as female ever since Chaucer.

As a vast collective project the *OED* is far from perfect, and inevitably so. But our complaints can only represent lovers' quarrels. I caught some vestiges of Victorian origins — references to the tools and habits of "African savages", for example. I found serious errors and omissions in the few words that I have studied intensely ('evolution' is badly served because the compiler did not grasp the full extent of pre-darwinian biological usage for the embryological theory of preformation). Some extinct words receive too much play (the complex terminological apparatus for Weismann's incorrect theory of heredity), while others now in vogue have not come to the *OED*'s attention (the far less complex terminology for evolutionary change in the timing of development).

This work needs no medals, no citations, no words of praise. It stands by and for itself, as a gift to all intellectuals. The truly great, in supreme and justified confidence, merely present themselves — *ecce homo, ecce res*. Christopher Wren has no gravestone, only a simple plaque on the floor of his greatest building, St Paul's Cathedral, engraved with the words: *si monumentum requiris, circumspice* (if you are searching for a monument, look around). For the *OED*, we need only say: *si monumentum requiris, inspicere* — look within. □

Stephen Jay Gould is a Professor in the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138, USA.

## Big business

Marco Festa-Bianchet

**Megaherbivores: The Influence of Very Large Body Size on Ecology.** By R.N. Owen-Smith. Cambridge University Press: 1988. Pp. 369. £40, \$64.50.

MEGAHERBIVORES are terrestrial herbivores that weigh over 1,000 kg: elephants, rhinos, hippos and giraffes. Biologically they are unique in several respects. For example, as adults they are almost completely beyond the reach of non-human predators. They also efficiently use low-quality forage; and because they need a lot of it, they have a great effect on ecosystems, and on the kinds and numbers of plants and animals that share their environment.

In his book devoted to these huge creatures, Owen-Smith first considers

their ecology, physiology and behaviour. In most cases, he presents a test of whether they are just especially large in size or are different from what may be predicted by extrapolating allometric relationships of smaller herbivores. He discusses the limitations inherent in the quality of the available data and in the statistics used to analyse them. Megaherbivores are difficult to study. Their longevity and slow reproductive rate, coupled with human exploitation and habitat destruction, cast uncertainty over the causes of particular ecological processes; for example, habitat fragmentation may prevent animals from dispersing and lead to severe habitat degradation. Perhaps in the past megaherbivores avoided this problem by moving to new areas. Exploitation by human beings probably affected population dynamics and habitat characteristics, so that it is sometimes unclear which changes in vegetation structure are a normal feature



of the community ecology of megaherbivores, and which result from the cessation of exploitation and sudden population increases in habitats whose structure changed while numbers were kept low.

The middle chapters of the book vary in scope. In some, for example those on nutrition, feeding ecology, reproduction and ecosystem processes, Owen-Smith can consider all megaherbivores as a group, and tests well-defined hypotheses. In others, such as those on behaviour and demography, differences between the species force less interesting species-by-species summaries of data. All chapters provide good literature reviews.

Most of the book is a prelude to two arguments presented at the end, where Owen-Smith puts forward explanations of the Pleistocene extinctions and discusses the conservation of surviving species.

Megaherbivores, once distributed over all continents, are now restricted to eight species in Africa and tropical Asia. Only hippos and giraffes appear to be safe over most of their range. Yet local problems of overpopulation show that "megaherbivores are embarrassingly successful when protected from human depredation".

The explanation for Pleistocene extinctions has human overexploitation as the main driving force, but takes into account climatic shifts and changes in habitat characteristics. Owen-Smith suggests that it was hunting that caused the demise of megaherbivores, especially in North America and Europe, and that changes in habitat (leading to extinction of some smaller species) were an effect and not a cause of their disappearance.

The chapter on conservation is excellent. Owen-Smith believes management is necessary for conservation, because artificial habitat fragmentation prevents dispersal of animals. He also recognizes that 'overgrazing' and 'overpopulation' are not biological realities, but reflect management choices. Protected from poaching, megaherbivores could recover to levels where light exploitation would be both feasible and desirable — but "without effective controls of illicit markets, populations of these great beasts will be driven inexorably towards extinction". □

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#### New in Britain

• *What Mad Pursuit: A Personal View of Scientific Discovery* by Francis Crick. Publisher is Weidenfeld & Nicolson, price is £12.95. For review see *Nature* 336, 268 (1988).

#### New in paperback

• *Bones of Contention* by Roger Lewin. Publisher is Penguin, price is £5.99. For review see *Nature* 330, 277 (1987).

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Defensive stare — detail from a bronze shield mount of the 2nd or 1st century BC found in the River Thames at Wandsworth. The picture is taken from Celtic Art by Ruth and Vincent Megaw, an illustrated survey of Celtic arts and crafts from 700 BC to AD 700. Published by Thames and Hudson, the book costs £28, \$45.

## On course for a better life?

F.E.G. Cox

**The Biology of Parasitism.** Edited by Paul T. Englund and Alan Sher. Alan R. Liss: 1989. Pp. 544. Hbk \$90, pbk \$45. Distributed in Britain by Wiley, hbk £68.85, pbk £35.50.

EACH summer since 1980, a small group of young scientists has gathered at Woods Hole to attend a course on advances in the molecular biology and immunology of parasites. The course is given by acknowledged experts from all over the world, and serves as a barometer of the state of the subject. In this volume, a number of the lecturers have attempted to convey its flavour and spirit to a wider audience. In all, 41 scientists have contributed 28 chapters, grouped into biological, immunological and molecular biological, biochemical and genetic aspects of parasitism, to produce a book that attempts to reflect the present state of our knowledge and to be an essential aid in undergraduate and postgraduate teaching.

The stated aim is to emphasize concepts rather than review research data, but sadly some of the authors have ignored this principle and the volume is thus very varied in content. Things get off to an excellent start with accounts of the global impact of parasitic diseases by Ken Warren, and of parasitic zoonoses by George Nelson, which set the scene. There are also specialized chapters on human schistosomiasis, amoebiasis, American leishmaniasis and trypanosomiasis and, towards the end, on toxoplasmosis, that are stimulating and make

provocative points.

The first SDS-PAGE gel appears on page 97, closely followed by the inevitable contributions on the surface antigens of *Plasmodium falciparum*, thick with facts and thin on concepts. The surface antigens of trypanosomes also receive considerable attention in three chapters; in one of them, John Donelson manages to concentrate on essentials and to ask interesting questions, as does Alan Sher in his contribution on vaccination. Fresh ground is explored by C.C. Wang, who considers new targets for chemotherapy, but his is the only contribution on this important area. Most of the remaining chapters are largely mini-reviews of topics covered in depth elsewhere.

Overall, this collection of articles represents a fair overview of present-day parasitological research and pin-points the unevenness of the subject's coverage. The important matter is the extent to which knowledge gleaned in the main laboratories of the world can be applied to the immense problems outlined by Ken Warren, and it is disturbing that most of the questions asked are of an academic rather than a practical sort. Equally disturbing is that scrutiny of the faces in the photographs of those who attended the last eight courses reveals that only three are black. One is tempted to ask whether parasites are important because they are the playthings of immunologists and molecular biologists or because they are daily life-threatening components of the environment for millions of people. Whatever the answer, the editors have produced a book that is useful, readable and well worth having. □

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# Measuring up the planet

Barry Parsons

**Geophysical Geodesy: The Slow Deformations of the Earth.** By Kurt Lambeck. Clarendon: 1988. Pp.718. Hbk £75, \$95; pbk £30.

GEODESY, which can claim to be the oldest of the geophysical sciences, maintains a somewhat different relationship to geophysics than the other geophysical sciences. The choice of *Geophysical Geodesy* as a title to emphasize that this book is about the geophysical applications of geodesy points to the usually dominant role of applied aspects of the subject. In contrast the title of a book on seismology would probably only be qualified if it were about exploration or engineering seismology.

This difference can be explained in part by the long timescales involved in the development of many geodetic techniques. Consider the example of very-long-baseline interferometry (VLBI), which is the method of determining the distance between two points on the Earth's surface by using radio telescopes at the two sites to measure the small differences in arrival time of signals from distant radio sources. It has taken 20 years of painstaking development for VLBI to get to the stage where it is possible to measure directly the relative motion between the tectonic plates. Although most geophysicists appreciate the potential of many geodetic measurements, their interest is only really aroused when it becomes clear that worthwhile information about the Earth will be forthcoming.

Fortunately for those interested in the geophysical applications of geodesy, in recent years several geodetic techniques have emerged that have either already demonstrated their value in studying the Earth or seem about to do so. Satellite altimeter measurements of the shape of the ocean surface have replaced direct measurements of gravity as the primary source of information about the gravity field over the oceans. These observations provide one of the few ways of looking at the effects of mantle convection beneath the oceanic lithosphere. In the next few years the Global Positioning System will become fully operational, and this method of measuring baseline lengths, which is similar to VLBI except that satellite radio sources rather than extraterrestrial ones are used, has the potential to determine exactly how crustal deformation in the tectonically active regions of continents takes place. The nature of the core-mantle boundary is the subject of much debate, and VLBI observations of the nutations of

the Earth's rotation axis allow estimates to be made of the departure of the ellipticity of the core-mantle boundary from the ellipticity expected due to the Earth's rotation alone.

Against this background, Kurt Lambeck has set himself the task of writing an account of the subject that encompasses not only the geodetic techniques but the geophysical applications as well. The breadth of material he covers is impressive, and the book will provide an excellent overview of the field for both students and research workers. Such a comprehensive treatment within a single volume has its drawbacks, however. The author has to assume that the intended audience of graduate students will already have been exposed to the basic ideas of both geodesy and geophysics, an assumption that will only be true for the more advanced

student. In addition, by introducing many results without the concomitant theoretical development, one loses a sense of the physics that lies behind the equations.

The subtitle, *The Slow Deformations of the Earth*, reflects the author's interests and the contents of the book are clearly weighted towards solid-Earth geophysics. Physical oceanography, which will be the principal beneficiary of the two satellite altimeter missions due over the next five years, appears in a subordinate role. Yet, with the broad range of geophysical applications that are included, and its appearance just as new observations are about to flood in, this book is bound to attract a wide audience. □

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## Today's data

Gunnar von Heijne

**Computational Molecular Biology: Sources and Methods for Sequence Analysis.** Edited by Arthur M. Lesk. Oxford University Press: 1988. Pp.254. £25, \$49.95.

FIVE years ago, in the typical molecular biology laboratory, there would be at least one computer whiz kid: a graduate student who stole time away from the bench to break into the central campus computer and entertain himself with adventure games. This student was often quickly assigned the more serious job of taking care of the sequence-analysis needs of the lab, installing the newly purchased software that no one else had found the time to get running. Thus everybody was happy: the student was promoted to the status of computer expert, and the 'real' scientists didn't have to soil their hands at the PC keyboard.

Times have changed. Even the staunchest 'wet' experimentalist has had to adapt to the fact that these days one simply cannot do molecular biology without occasional recourse to the help of a computer. This, of course, is mainly a result of the extraordinary growth of DNA sequence data over the past ten years: more than 20 million bases worth of sequence is now stored in the main data banks such as GenBank or the European Molecular Biology Laboratory's data library, and only computers can deal with such massive amounts of information.

The world of 'machine molecular biology' is not immediately accessible to the average biologist, however, and good introductory texts are badly needed. The so-called CODATA Task Group on Coordination of Protein Sequence Data

Banks has now put out a volume called *Computational Molecular Biology*, edited by Arthur Lesk, to meet the demand.

The book is organized around four questions — What data are available? How can one gain access to them and to the necessary programs? What calculations can be done? And how can the results of the calculations be intelligently and cautiously interpreted? The first and second questions are treated at length; the third and fourth are less fully explored. We thus learn a lot about a number of DNA- and protein-sequence data banks around the globe, and we are introduced to the hardware and software of sequence analysis as well as to some aspects of computer networking. But we are told less about the actual methods and algorithms that allow the computer to produce the output we want.

Lesk apparently sensed this bias at an early stage, and has written a number of the less 'computerish' chapters himself, chapters for people who care more about how to find probable coding regions, or predict the structure of a protein, than about the details of how a GenBank entry is organized. Lesk has also come up with a couple of remarks that I will cherish for a long time to come. Thus, in summing up secondary structure prediction for proteins, he delivers the following: "Running a secondary structure prediction on a newly-determined sequence just because everyone else does so, is to be deplored, and the fact that the results of such predictions are generally ignored is insufficient justification for doing and publishing them". This is the sort of text that should be printed on every sequence-analysis software diskette as a 'Warning from the Surgeon General'. □

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# The search for the causes of breast and colon cancer

Walter Willett

Epidemiological studies of breast and colon cancers implicate diet as a causative factor but the evidence is stronger for colon cancer, the occurrence of which may be reduced by diets with less animal fat and more fruit and vegetables.

AMONG non-smokers, cancers of the breast and colon are the most important malignancies in Western societies. By the age of 75 years, over eight per cent of women in the United States will develop breast cancer and about three per cent of both sexes will be diagnosed as having colon cancer<sup>1</sup>. Another one to two per cent of men and women will develop rectal cancer by the time they are 75; although this is sometimes not distinguished from colon cancer, it will not be considered here because some of its epidemiological features are distinct. Treatment of breast and colon cancer remains disappointing. Hence, considerable interest has focused on determining the causes of these cancers, with the hope that such knowledge will lead to practical means of prevention.

The occurrence of both breast and colon cancer is strongly associated with modern affluence, suggesting that they may share important aetiological factors. Indirect evidence, largely based on differences in cancer rates between countries and changes in rates among migrants, has implicated diet as potentially important, but specific aspects of diet have not yet been definitively identified from epidemiological investigations. Much of the available information is derived from case-control studies in which reports from cancer patients about their past dietary practices are compared with those of persons without cancer. The possibility of biased reporting of former diets in these studies is difficult to eliminate. Prospective cohort studies, in which dietary variables measured among a large number of people are related to subsequent risks of cancer, should provide more dependable data, but these are only beginning to be available because it takes time to accumulate enough cancer cases. The existing limited data suggest that, despite gross similarities in the epidemiology of breast and colon cancer, incidence of the colon cancer is likely to be more responsive to dietary change and thus be easier to prevent.

## Roles of environment and genetics

Evidence is strong for a substantial genetic contribution to the risk of breast and colon cancers. Having a first-degree relative with breast cancer increases risk by about 1.5–3 fold<sup>2,3</sup>. Although this familial association could conceivably be due to a shared environment, the much higher risks (approximately tenfold) associated with some familial patterns, such as having a mother or sister with bilateral breast cancer at a young age, makes it more likely that the familial associations are genetically based. Even one case of colon cancer in a first-degree relative increases the risk for other family members by approximately threefold<sup>4,5</sup>.

Nevertheless, genetic factors are not the only explanation. Rates of breast and colon cancer vary strikingly between countries, by five to tenfold<sup>6</sup>, and migrants moving from low- to high-risk countries adopt the rates of the new country<sup>7,8</sup>. Furthermore, there have been large increases in rates in some countries during this century<sup>9,10</sup>. Thus, both breast and colon cancer almost certainly involve a strong interaction of genetics and environment. Without the necessary environment, few will develop these diseases; but in a suitable environment, those with a genetic predisposition will be at high risk. Aetiological

studies of breast and colon cancer will become far more powerful when specific genetic markers for predisposition become available.

Differences in rates between countries have been used to estimate that 90% of colon and 80% of breast cancers in the United States of America can be attributed to non-genetic factors<sup>11</sup>. The challenge is to identify the specific environmental factors. Although there is a plethora of hypotheses, well established risk factors are few and do not account for the major international differences or lead directly to practical preventive measures. Diet is a prime candidate: Doll and Peto<sup>11</sup> have suggested that as much as 50% of breast and 90% of colon cancer in the United States might be prevented by changes in diet. Before examining specific dietary hypotheses, the better established risk factors will be briefly discussed.

## Risk factors for breast cancer

As early as 1700, Ramazzini<sup>12</sup> observed that child-bearing reduces the risk of breast cancer. In an international study, MacMahon *et al.*<sup>13</sup> found that the dominant protective factor was an earlier age at first birth, rather than the number of children: a first birth after the age of 35 years increased the risk about threefold compared with a first birth before the age of 20. Other data suggest that having a larger number of children also independently reduces the risk<sup>14,15</sup>. The protective effect of early first full-term pregnancy may be due to a permanent differentiation of mammary stem cells, resulting in a reduced lifetime risk of breast cancer<sup>16,17</sup>.

Many studies show an association between early age at menarche and increased risk of breast cancer; typically early menarche confers about a 1.5–2 fold increase in risk compared with late menarche<sup>14,18,19</sup>. Early menopause provides protection<sup>14,20</sup>; removal of both ovaries before the age of 35 reduces the risk of disease by about 0.6 compared with natural menopause. These associations with reproductive factors provide support for a hormonal role in the aetiology of the disease<sup>21,22</sup>.

The relationship between sex hormone levels in blood or urine and risk of breast cancer have been examined in many studies but clear and consistent findings have not emerged. All these studies have been small, however, and most have been conducted among patients who already have cancer, who may have had altered hormone levels because of the disease. Key and Pike<sup>23</sup> concluded in a recent review that the data overall suggest a promoting effect of oestrogens and possibly also of progestagens.

The possible role of sex hormones in the development of breast cancer has raised concerns that oral contraceptives and post-menopausal replacement hormones might increase the incidence of this disease. Overall, little relationship has been seen between oral contraceptive use and breast cancer<sup>24</sup>, but longer-term use before a first full-term pregnancy may elevate risk<sup>24,25</sup>; more data are needed to resolve this important issue. In most studies, oestrogen-replacement therapy after menopause has not been associated with the incidence of breast cancer<sup>26</sup>, but use of hormones for several decades may cause a modest

increase<sup>27</sup>. Most investigations have concentrated on past users and the possibility that current use may promote tumour growth has generally not been addressed.

Ionizing radiation, including that of X-rays, is a well-documented cause of breast cancer, especially for exposure during adolescence<sup>28,29</sup>, but this cannot account for a large proportion of the disease because not many women receive a significant exposure. Benign lesions are probably best considered as pre-malignant responses to primary aetiological factors: the subsequent risk of breast cancer is strongly related to the degree of proliferative change and atypia found in biopsies of such lesions<sup>30</sup>.

### Risk factors for colon cancer

Few specific risk factors for colon cancer have been established. Inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, greatly increase the risk<sup>31</sup> but because of their rarity, these account for a very small proportion of cases. The rare familial polyposis syndromes are associated with high risk<sup>31</sup>, but also contribute only slightly to the overall incidence of colon cancer.

The presence of adenomatous colon polyps, which can be considered as precursor lesions<sup>32</sup>, is associated with an elevated risk of colon cancer. Because the polyps can be examined directly by endoscopy, they provide a way of studying the progression to colon cancer that does not exist for breast cancer.

### Diet and cancers of the breast and colon

**Dietary fat and breast cancer.** For the last decade, the dominant aetiological hypothesis for breast cancer has been that high fat intake, and animal fat in particular, is the primary cause of the large differences in rates between countries<sup>33</sup>. This notion is based largely on two lines of evidence: striking international correlations (approximately 0.80) between *per capita* consumption of fat and breast cancer rates<sup>8</sup>; and animal experiments in which diets high in fat increase the occurrence of mammary tumours. Subsequent evidence has not, overall, supported this hypothesis.

It is tempting to draw causal inferences from the international correlations, but these studies provide only weak evidence because the actual causes might involve many other factors related to economic development. Factors such as physical activity, reproductive variables, body composition at different times in life and energy balance are particularly difficult to measure and control in correlational analyses. A recent survey of cancer rates in 65 rural counties in China with similar low levels of economic development<sup>34</sup> strongly suggests that the international comparisons are seriously confounded by factors related to industrialization: fat intake ranged from 5–47% of energy but breast cancer rates did not vary with fat intake and were at most about one-tenth of those in the United States.

Case-control studies and prospective cohort studies provide more opportunity to measure and adjust for other factors that may be related to risk of cancer. For breast cancer, no material association with fat intake has been found in case-control studies from New York<sup>35</sup>, Hawaii<sup>36</sup>, Australia<sup>37</sup>, Greece<sup>38</sup> and Japan<sup>39</sup>. Although a study from Canada was originally reported as showing a positive association<sup>40</sup>, it was not statistically significant and examination of the data indicates that the average fat intake reported by cases was virtually identical to that reported by controls. In other case-control studies, only a limited list of foods was included<sup>41–46</sup>; some have construed sporadic associations with foods containing fat as evidence that fat causes breast cancer. These data are difficult to interpret because of the tendency to focus on foods within a study for which associations are seen and because other aspects of the diet, including total energy intake, cannot be controlled for.

The potential for distortion of associations because of differential recall of past diet is inherent in case-control studies but is eliminated in prospective investigations. Only two pros-

pective studies with a comprehensive assessment of diet have been published. In the larger, based on a dietary questionnaire completed by 89,538 registered nurses in the United States in 1980<sup>47</sup>, weak inverse associations with incidence of breast cancer were seen for total, saturated, and polyunsaturated fat. In the other, there was a statistically significant protective effect of total dietary fat but this inverse relationship was weaker and no longer significant after controlling for total energy intake<sup>48</sup>. In prospective studies with limited dietary data, a positive association was seen with meat intake in Japan<sup>49</sup> but not among Seventh-Day Adventists in California<sup>50</sup>. In the latter, breast cancer rates tended to increase, rather than decrease, the longer a Seventh-Day Adventist vegetarian diet, which contains substantially less animal fat than the general diet of North Americans<sup>51</sup>, was consumed.

Findings from case-control and prospective cohort studies, which generally fail to support the dietary fat hypothesis, have been criticized on the basis that diets in the populations studied are not sufficiently heterogeneous and the methods for measuring diet are not accurate enough to detect potential associations<sup>52,53</sup>. But even when the degree of measurement error associated with the dietary questionnaire used in the larger of the two prospective studies is accounted for<sup>47</sup>, the data are compatible with only a very weak positive association with fat intake<sup>54,55</sup>. Viewed another way, if the positive associations suggested by the international data were true, the inverse associations actually seen in the two prospective studies would be statistically extremely unlikely.

The published case-control and cohort studies cannot exclude the possibilities that reducing fat intake to well below 30% of total energy intake, the level now frequently recommended<sup>33,56</sup>, or at an earlier period in life, might influence breast-cancer rates. No appreciable reductions in breast-cancer risk among vegetarian nuns<sup>57</sup> and Seventh-Day Adventists<sup>58</sup> have, however, been observed, which argues against an important influence of animal fat over several decades.

The relationship between fat intake and the occurrence of mammary tumours in animals, which cannot be reviewed in detail here, remains controversial<sup>59,60</sup>. Restricting energy intake dramatically lowers the incidence of mammary tumours. As fat is uniquely dense in energy, a low-fat diet also tends to be low in energy unless the available energy intake is carefully controlled. Thus, rats on a low-fat *ad libitum* diet have lower tumour incidence than those on a high-fat *ad libitum* diet; in one typical study<sup>61</sup>, a reduction of 40% was seen. In the same study, however, when the high-fat diet was restricted to provide 20% less total energy intake, tumour incidence decreased by 90%.

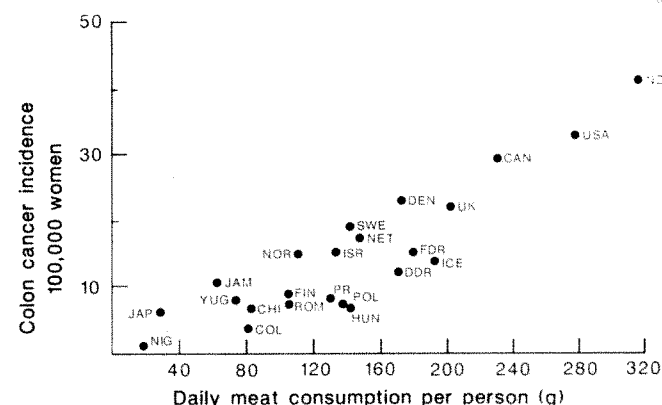


FIG. 1 Correlation between intake of meat per person and the incidence of colon cancer among women in 23 countries<sup>6</sup>. The strong positive association suggests that animal fat or protein may increase the risk of colon cancer. Because the countries with high meat intake and colon cancer rates tend to be affluent Western nations that differ in many ways from the countries with low meat intake and colon cancer rates, these data are far from conclusive.

Albanes<sup>62</sup> has performed a meta-analysis of the experiments on diet and mammary cancer in mice over the past 50 years, in which he finds an extremely strong overall positive association for total energy intake. After adjustment for total energy intake, the fat composition showed a weak inverse relationship to incidence of mammary tumours. In another review of animal experiments, Birt<sup>59</sup> concluded that there is evidence for an effect of dietary fat which is independent of total energy intake.

Is any particular rodent model relevant to human breast cancer? The models usually involve a potent carcinogen or exposure to a mammary tumour virus, extreme contrasts in diet, and observations for relatively short periods before menopause. In a recent study<sup>63</sup> that more closely simulated the human experience, the occurrence of spontaneous mammary adenocarcinomas was investigated among more than 5,000 female rats and mice randomly assigned to either a 10% fat diet or corn-oil supplements that increased fat to 30% of calories. Even though the animals fed corn oil gained more weight than those on low-fat diets, the two-year cumulative incidence among rats was 2.5% on low-fat and 1.5% on high-fat diets. Among mice, the corresponding incidence rates were 1.7% and 1.3%. A case-control study of breast cancer in dogs, which was conducted by interviewing owners about the usual foods consumed by their animals<sup>64</sup>, revealed an extremely wide variation in dietary fat composition but no association with risk of breast cancer. A consensus clearly does not exist at present that the total fat composition of the diet in animals influences the risk of mammary tumours, so laboratory data cannot be used as an argument for or against any relationship in humans.

The plausibility of the fat-and-breast-cancer hypothesis would be greatly enhanced if it could be shown that fat influences a biochemical mediator of breast cancer, but unfortunately no such mediator has been well established. Blood or urinary oestrogen levels are the most promising candidates, but the relationships between diet and hormone levels are unclear at present<sup>65-67</sup>.

**Dietary fat and colon cancer.** In contrast to breast cancer, epidemiological studies provide some, but not conclusive, evidence that animal fat or meat intake is associated with risk of colon cancer. In comparisons between countries, rates of colon cancer are strongly associated with intake of animal fat and meat (Fig. 1). High total fat intake has been associated with increased risk of colon or rectal cancer in case-control studies from Canada<sup>68</sup>, Australia<sup>69</sup>, Utah<sup>70</sup>, England<sup>71</sup>, New York State<sup>72</sup> and eastern Australia<sup>73</sup>, although the last study is suspect as data from controls were not collected concurrently. In similar studies from southern France<sup>74</sup>, Paris<sup>75</sup> and Belgium<sup>76</sup>, however, no association was found. In the French and Belgium studies, intake of vegetable fats was inversely related to risk of colon cancer. The numbers of colon cancers in prospective studies have been relatively small. Among Japanese-Hawaiian men, those with the highest fat intake had a reduced risk of colon cancer<sup>77</sup> and among Chicago men no association was found between fat intake and colon cancer<sup>78</sup>. In preliminary reports, animal fat intake was positively associated with risk of colon or large bowel cancer among the 89,538 women in the Nurses' Health Study<sup>79</sup> and among 35,000 Seventh-Day Adventists<sup>80</sup>.

In case-control studies of diet and colon cancer, there is a notably consistent positive association with total energy intake<sup>68-72</sup>. This is particularly puzzling because higher energy expenditure seems to reduce risk of colon cancer<sup>81,82</sup> and obesity is unrelated, or at most weakly associated, with this disease (see below). The positive association with total energy intake has important implications for interpreting data on specific nutrients, because all tend to be correlated with total energy intake<sup>83</sup>. It is important to adjust statistically for total energy intake in the analysis of these studies to distinguish between the effect of overall food intake and that of dietary composition, especially for nutrients such as dietary fat that are highly correlated with energy intake. Individuals must alter their intake of

specific nutrients primarily by manipulating the composition rather than the total amount of food eaten, unless they are to alter substantially their physical activity or weight. Thus, the intake of nutrients adjusted for total energy intake is most informative for making decisions about changes in diet. The association of dietary saturated fat with colon cancer was independent of total energy intake in one study<sup>84</sup> but not in another<sup>69</sup>; most other case-control studies have not evaluated whether the observed association with overall fat intake is independent of energy intake.

Plausible mechanisms exist for an influence of dietary fat on the incidence of colon cancer; higher fat intake increases excretion of bile acids and the growth of colonic bacteria capable of converting bile acids to carcinogenic substances<sup>85,86</sup>.

### Dietary fibre

Burkitt's hypothesis<sup>87</sup> that dietary fibre (non-digestible complex carbohydrates) reduces the incidence of colon cancer seems to be, at best, an over-simplification. Increased intake of fibre is thought to reduce the transit time for faecal matter in the gut, and to dilute potential carcinogens in the faeces<sup>88</sup>. High intake of fruits and vegetables has been consistently related to lower risk of colon cancer but consumption of cereal products has not. Although countries where there is a lower intake of fibre tend to have higher rates of colon cancer<sup>89</sup>, inverse associations have been seen in some<sup>70,72,73,76</sup> but not all<sup>68,74,69,71,75</sup> case-control studies.

Some of the inconsistency may result from inadequate adjustment for total energy intake, for even the direction of association can depend on whether energy intake is appropriately considered in the data analysis<sup>70</sup>. Evidence that grain products are protective is considerably weaker than it is for fruits and vegetables; in all eight studies in which the sources of fibre were examined separately, grain fibre or cereal intake was either unrelated or positively associated with risk of colon cancer, whereas intake of fibre from fruits and vegetables was protective<sup>69,74,82,90-94</sup>. A protective effect of fruits or vegetables was also found in two additional case-control investigations<sup>95,96</sup>.

Although the evidence that fruits and vegetables are protective is strong, the specific foods related to reduced risk are poorly defined and the responsible factors are unknown. Specific fractions of fibre may be the active agents; other candidates include vitamins, such as C or E, protease inhibitors<sup>97</sup> and indoles<sup>98</sup>. In one analysis, no association was found with  $\beta$ -carotene<sup>99</sup>, whereas an inverse relationship was seen in another<sup>92</sup>.

More limited evidence indicates that some vegetable factor may be protective for breast cancer, which was found to be inversely related to vegetable intake among women in Greece<sup>100</sup> and to total vitamin A intake, largely from vegetable sources, among women in New York State<sup>101</sup>. This possibility requires further examination.

### Alcohol

Rapidly accumulating evidence, summarized elsewhere<sup>102</sup>, indicates that intake of alcoholic beverages is associated with a higher risk of breast cancer (Fig. 2). Positive associations with beer, wine and liquor in various populations indicates that the effect is due to alcohol *per se*<sup>103</sup>. The possibility cannot be entirely excluded that some other characteristic of women who drink alcohol might explain this association, but such a characteristic would have to cause a larger attributable fraction of breast cancers than the known risk factors and also be strongly associated with alcohol use. When risk factors for breast cancer were allowed for, the association with alcohol was not materially altered in any of the published studies. If the association is truly one of cause and effect, possible interactions with other risk factors may be important. In one case-control study<sup>104</sup>, only alcohol consumed before the age of 30 years contributed to increased risk. If confirmed in other studies, this would mean that if a middle-aged woman reduced her alcohol consumption,



it would not alter her risk of breast cancer.

In a recent cohort study there were higher rates of colon cancer among regular drinkers, especially women, than among non-drinkers<sup>105</sup>. Further prospective data are needed that clearly distinguish colon from rectal cancers, for which the data implicating alcohol are somewhat stronger. A weak positive association, reviewed elsewhere<sup>105</sup>, between use of alcoholic beverages and risk of colon cancer has been observed sporadically in case-control studies.

### Other specific dietary factors

Many other dietary factors have been postulated as either protective or causative for breast and colon cancer. Putative protective factors include selenium<sup>106</sup>, vitamins C<sup>107</sup> and E<sup>108</sup>, preformed vitamin A and its precursor,  $\beta$ -carotene<sup>109</sup>; vitamin D<sup>78</sup>, naturally occurring inhibitors in plants such as the indoles<sup>98</sup>, protease inhibitors<sup>97</sup> and fish oils<sup>110</sup>. Other causative factors suggested include specific fatty acids, such as the *trans*-isomers produced in processing vegetable oils<sup>111</sup>, and mutagenic products formed in the cooking of food<sup>112</sup>. Calcium may reduce risk of colon cancer<sup>113</sup>; reduced colonic epithelial-cell proliferation has been reported in subjects supplemented with calcium carbonate<sup>114</sup> and there is some epidemiological evidence<sup>78,115</sup>. Data are at present too limited either to refute or to confirm any of these hypotheses with confidence.

### Energy balance

In animals, restricting energy intake reduces the occurrence of mammary and colon tumours and other neoplasms<sup>60-62,116,117</sup>; accumulating evidence suggests that reduced energy intake, particularly early in life, may also reduce the incidence of breast cancer in humans. The influence of energy intake on cancer risk in humans is best studied using physical measurements that reflect energy balance at different periods in life. Substantial restriction of energy intake before the age of 20 will stunt height. Among adults, weight standardized for height and measures of change in weight are sensitive indicators of long-term energy balance.

Internationally, the high correlation (0.8) between the average height of adult women in a country and national rates of breast cancer<sup>118</sup> provides some evidence for a protective effect of energy restriction before adulthood. Positive associations have been found between height and risk of breast cancer in several countries that experienced periods of food shortage, such as Greece<sup>119</sup>, the Netherlands<sup>120</sup> and Japan<sup>121</sup>. In the United

States<sup>122</sup> and Scandinavia<sup>123</sup>, height is generally unrelated to breast cancer, compatible with a sufficient supply of energy for most individuals to attain their genetically determined maximum height. In a recent sample of women from the United States,<sup>124</sup> that was enriched in individuals at increased risk of malnutrition, a positive association between height and risk of breast cancer was found<sup>124</sup>.

The relationship between obesity and risk of breast cancer is complex. In a large prospective study in the United States, mortality from breast cancer was about 50% higher among women who were more than 40% above average weight for their height than among women of average weight<sup>125</sup>. This may partly be because tumours are diagnosed later among obese women<sup>122</sup>, rather than because of an aetiological role of obesity. In studies that considered the incidence of breast cancer rather than mortality, a distinct difference has been found between pre- and post-menopausal women. Before menopause, greater relative weight is associated with a reduced risk of breast cancer<sup>122,126</sup>; rates among those in the heaviest 20% are about 40% lower than among the thinnest. Among post-menopausal women, positive associations with relative weight have been seen in several studies<sup>127-131</sup>, but these have been generally weak, without a clear dose-response relationship, and in some studies no association was observed<sup>126,132</sup>.

A weak positive association was seen between relative weight and mortality from colon cancer in men, but not women, in one large prospective study<sup>125</sup>. However, in some case-control<sup>68</sup> and prospective cohort<sup>133,134</sup> studies, relative weight had little association with incidence of colon cancer.

### Contrasts between breast and colon cancers

Although the striking international correlations of both breast and colon cancer rates with indices of affluence suggest common aetiological factors, other evidence indicates important differences in aetiology or the period of life during which the factors act. For example in the United States, Japanese immigrants have rates of colon cancer similar to those of the caucasian population, whereas rates of breast cancer take several generations to approach those of the resident population<sup>135,136</sup>. Rates of breast cancer among Seventh-Day Adventists are similar to those in the general population, but rates of colon cancer are about 40% lower<sup>58</sup>.

The relationship between changes in diet and rates of mortality from breast and colon cancer are uniquely illustrated in Japan, where there has been a dramatic change in diet since World War II (Fig. 3). Between 1955 and 1975, fat intake increased from about 10% to about 25% of total energy intake. This has been accompanied by a striking rise in mortality from colon cancer but only a small change in breast cancer mortality. These data do not necessarily indicate that the fat composition of the diet caused the rapid rise in colon cancer, but they do suggest strongly that environmental changes will more rapidly influence rates of colon than breast cancer and that large changes in fat intake will be accompanied by changes in rates of breast cancer that are, at most, modest or substantially delayed.

It is useful to compare case-control and prospective cohort studies of colon and breast cancer conducted by the same investigators using similar methods, because the results are relatively unobscured by the differences typically found in study populations and research designs. Such pairs of studies have been conducted in upstate New York<sup>96,101</sup>, Greece<sup>93,100</sup>, Canada<sup>68,40</sup>, Californian Seventh-Day Adventists<sup>41</sup>, Australia<sup>69,37</sup> and nurses in the United States<sup>47,79</sup>. Except in the upstate New York study, clear associations were seen between fat or meat intake and risk of colon cancer, but not of breast cancer, in each pair of analyses. These comparisons provide further evidence that the failure to find associations between diet and breast cancer in case-control and cohort studies is not simply due to lack of heterogeneity in diet or imprecise methods of assessing diet.

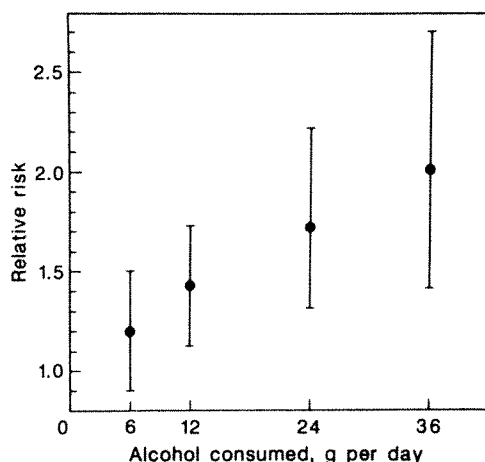


FIG. 2 Risk of breast cancer related to level of alcohol consumption, with 95% confidence intervals, based on pooled estimates from five prospective studies<sup>102</sup>. In women consuming 12 g of alcohol per day, about one standard drink of beer, wine or liquor, the risk of breast cancer was ~40% higher than in non-drinkers. The risk for 24 g per day rose to ~70% and for 36 g per day to ~100% compared with non-drinking women. (From ref. 111, with permission of the American Medical Association.)

## Prospects

In spite of strong evidence that non-genetic factors account for the high rates of breast and colon cancer in industrialized countries, few specific aetiological factors are well established that offer the potential for prevention. Among the associations considered here, drinking alcohol and the risk of breast cancer is the relationship most strongly supported by epidemiological findings, but it is not certain whether this represents cause and effect. The benefits of reducing alcohol intake remain unclear because any influence on breast cancer risk may be limited to young adulthood. In adult women, a modest reduction in dietary fat and avoiding obesity seem likely to have little, if any, impact on reducing the risk of breast cancer. On the contrary, accumulating data indicate that being thin before menopause may actually increase the risk of breast cancer.

Several lines of evidence indicate that risk of colon cancer may be more responsive than breast cancer to changes in diet. Associations between dietary fat and colon cancer have been found in several studies; whether this represents a causal effect of the fat composition of the diet remains unproven and the type of fat rather than its total amount may be most important. Inverse associations have been found repeatedly between consumption of fruits and vegetables and the risk of colon cancer. The process of translating these epidemiological findings into public recommendations has taken an interesting turn: data on fruit and vegetable consumption have been used to compute fibre intake and, based on the resulting inverse associations with fibre intake, recommendations have been made to consume more whole-grain cereal, even though there is a consistent lack of evidence for a protective role of cereal fibre in epidemiological studies.

Except for limiting alcohol intake, which could at most only modestly decrease the incidence of breast cancer, prospects for prevention of breast cancer seem limited in the near future. The potential for preventing colon cancer by changes in diet seems greater, but exactly how needs to be more clearly defined. Increasing the intake of fruits and vegetables seems promising and reducing consumption of animal fats may prove to be effective. Large prospective studies of diet that are now under-

way or planned, together with randomized trials of dietary factors in relation to recurrence of colon polyps that are now in progress, will provide much additional information about the effects of specific foods and nutrients. □

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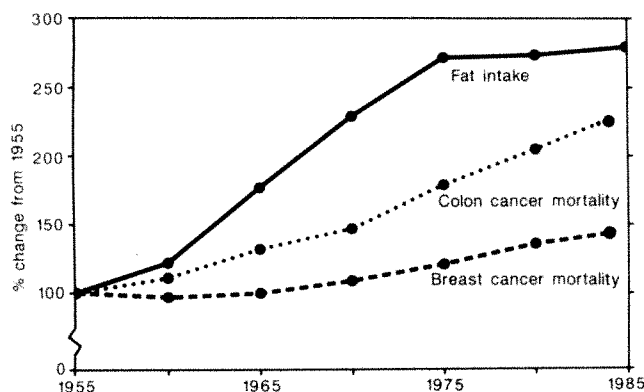


FIG. 3 Relative changes in fat intake and age-adjusted rates of mortality from breast and colon cancer in Japan between 1955 and 1985. During this period, fat intake increased by ~180% (from ~10-25% of energy intake) whereas total energy intake remained nearly constant. By 1984, age-adjusted mortality from breast cancer had increased ~45%, whereas mortality from colon cancer among women increased by 130%, an increase proportional to increased fat intake with a lag of ~15 years. Much of the modest increase in breast cancer mortality is probably a result of marked changes in reproductive patterns; for example, the percentage of women having their first child before the age of 19 decreased from 13 to 4% between 1950 and 1975<sup>10</sup>. Although data for incidence of breast cancer are available for a more limited period, they also indicate only a slow increase over time<sup>135</sup>. Absolute levels in 1955 were 20.3 g per day of fat per capita, 1.9 colon cancer deaths per 10<sup>5</sup> person-years, and 3.3 breast cancer deaths per 10<sup>5</sup> person-years. (Based on data from Health and Welfare Statistics Association, Japan<sup>11</sup>.)

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## ARTICLES

# The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis

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Three of the four yeast ubiquitin genes encode hybrid proteins which are cleaved to yield ubiquitin and previously unidentified ribosomal proteins. The transient association between ubiquitin and these proteins promotes their incorporation into nascent ribosomes and is required for efficient ribosome biogenesis. These results suggest a novel 'chaperone' function for ubiquitin, in which its covalent association with other proteins promotes the formation of specific cellular structures.

THE covalent ligation of ubiquitin to various acceptor proteins in eukaryotic cells participates in or regulates a number of cellular processes, such as selective protein degradation<sup>1-3</sup>, DNA repair<sup>4</sup>, progression through the cell cycle<sup>2,5,6</sup> and a variety of stress responses<sup>7-11</sup>. A major function of ubiquitin is to mark

proteins destined for selective elimination<sup>12-14</sup>. An alternative role for ubiquitin has also been suggested<sup>15</sup>, in which it reversibly joins to an acceptor protein to modulate protein function without metabolically destabilizing the acceptor protein. *In vivo* acceptors of ubiquitin include histones H2A and H2B<sup>16</sup>, actin<sup>17</sup>, the lymphocyte homing receptor<sup>18</sup>, the platelet-derived growth factor receptor<sup>19</sup>, the growth hormone receptor<sup>20</sup> and the intracellular neurofibrillary tangles in neurodegenerative diseases such as Alzheimer's<sup>21-22</sup>.

The conjugation of ubiquitin to other proteins involves the formation of an isopeptide bond between the C-terminal glycine residue of ubiquitin and the  $\epsilon$ -amino group of a lysine residue in the acceptor protein<sup>1,12</sup>. In the yeast *Saccharomyces cerevisiae*, the six or more enzymes that catalyse such reactions<sup>15</sup> include the products of the genes *RAD6*, which is required for DNA repair<sup>4</sup> and *CDC34*, which is required for the transition from the G1 to the S phase of the cell cycle<sup>5</sup>. Ubiquitinated proteins often contain single ubiquitin moieties linked to one or more of the acceptor protein's lysine residues<sup>12,16,17,23</sup>. Alternatively, several ubiquitin moieties can be attached sequentially to an acceptor protein to form a chain of branched ubiquitin-ubiquitin conjugates in which the C-terminal Gly 76 of one ubiquitin is

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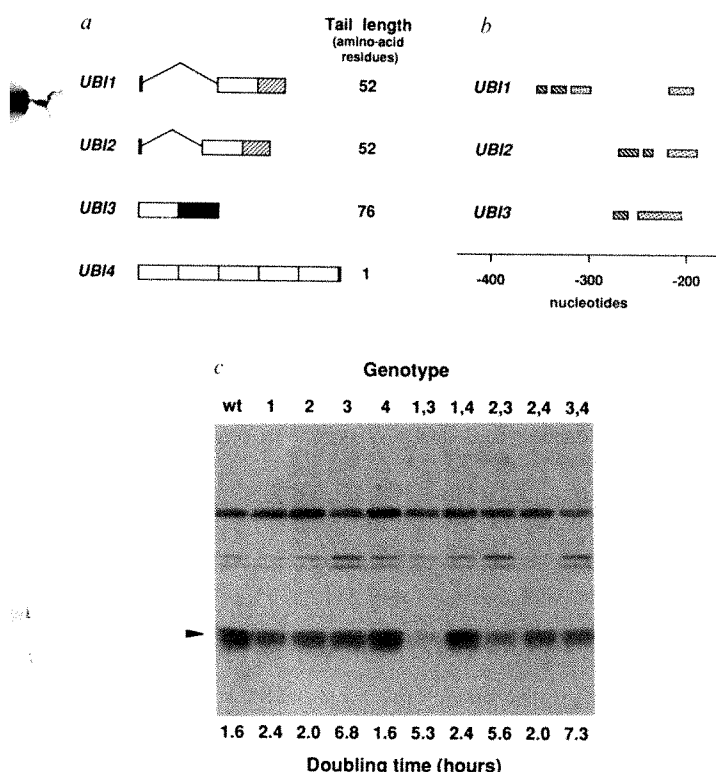


FIG. 1. *S. cerevisiae* ubiquitin genes and ubiquitin mutants. **a**, Organization of yeast ubiquitin genes. Open blocks represent the 228-base pair (bp) ubiquitin-coding repeats. Striped and shaded blocks represent tail-coding elements. *UBI1* and *UBI2* contain identically positioned, mostly nonhomologous introns within their ubiquitin-coding elements<sup>24</sup>. **b**, Putative upstream activation (UAS) sites of *UBI1-UBI3* genes. Positions are relative to the ATG start codon in each gene. Striped blocks represent near-matches (11–14 out of 15) to the consensus sequence of UAS sites within genes for ribosomal proteins<sup>30</sup>. Stippled blocks represent T-rich stretches often found downstream of such UAS sites<sup>30</sup>. **c**, Immunoblot analysis of intracellular ubiquitin levels and doubling times of yeast strains bearing deletions of various ubiquitin genes. Wild-type lane is on the left; other lanes are numbered at the top according to the ubiquitin gene or genes deleted in the corresponding strain (for example, lane 1, *ubi1* strain). The ubiquitin band is indicated by an arrow. The doubling time of each strain at 30 °C in YPD, a rich medium<sup>46</sup>, is indicated underneath; the same growth conditions were used in the immunoblotting experiment. The bands of high molecular weight presumably correspond to ubiquitin-protein conjugates.

**METHODS.** Exponentially growing cells were centrifuged at 5,000 *g* for 5 min at 4 °C, resuspended in 30 mM Tris-HCl (pH 6.8), 1 mM MgCl<sub>2</sub>, centrifuged again, and resuspended in a buffer containing 30 mM Tris-HCl (pH 6.8), 1 mM MgCl<sub>2</sub>, 2 mM *N*-ethylmaleimide, 10 µg ml<sup>-1</sup> antipain, 10 µg ml<sup>-1</sup> leupeptin, 10 µg ml<sup>-1</sup> aprotinin, 10 µg ml<sup>-1</sup> chymostatin and 10 µg ml<sup>-1</sup> pepstatin (Sigma). Cells were lysed by vortexing in the presence of glass beads, and the extracts were centrifuged at 12,000 *g* for 10 min. Protein (150 µg) was loaded onto each lane of an 18% polyacrylamide-SDS gel. Fractionated proteins were then electroblotted onto PVDF membranes (Millipore) for 20 min at 13 V cm<sup>-1</sup> (under these conditions, the transfer of high molecular weight proteins is not complete). Filters were probed with an antibody to ubiquitin<sup>21</sup>, followed by <sup>125</sup>I-labelled Protein A (ICN Radiochemicals) using standard techniques<sup>8</sup>. The filter-bound <sup>125</sup>I-Protein A was detected by autoradiography. Doubling times were determined by monitoring the absorbance at 600 nm of exponentially growing liquid cultures. All strains analysed were *MATα*. All strains used in this work are congeneric, being derived from DF5 (*MATα/MATα*, *trp1-1* (*am*)/*trp1-1* (*am*), *ura3-52/ura3-52*, *his3-Δ200/his3-Δ200*, *leu2-3,2-112/leu2-3,2-112*, *lys2-801/lys2-801*), a previously described homozygous diploid<sup>8</sup>. Precise chromosomal deletions were constructed essentially as described<sup>8</sup>, except that in the case of the *UBI2* *in vitro* deletion, we used restriction endonucleases rather than an oligonucleotide. A 1.6 kilobase (kb) genomic *HincII* fragment containing the *UBI2* gene<sup>24</sup> was subcloned into pUC13 (ref. 48), and the resulting plasmid was digested with *HindIII* and *ClaI*, deleting all but 8 bp of the *UBI2* coding sequence and no noncoding sequence. Subsequent steps were as described<sup>8</sup>. The deletion-disruption mutation *ubi1* was marked with the *TRP1* gene, whereas *ubi2* was marked with *URA3* and *ubi3* with *HIS3*.

joined to the internal Lys 48 of an adjacent ubiquitin<sup>23</sup>. The attachment of such a multi-ubiquitin chain to the proteolytic substrate is apparently essential for the degradation of a variety of proteins<sup>23</sup>.

Unlike the branched ubiquitin-protein conjugates, which are formed post-translationally, linear ubiquitin adducts are formed as the translational products of natural gene fusions<sup>7,24,25</sup>. Thus, in *S. cerevisiae* for example, ubiquitin is generated exclusively by proteolytic processing of precursors in which ubiquitin is joined either to itself, as in the linear polyubiquitin protein *UBI4*, or to unrelated 'tail' amino-acid sequences, as in the hybrid proteins *UBI1*, *UBI2* and *UBI3* (Fig. 1a). In growing yeast cells, most ubiquitin is generated from the *UBI1-UBI3* hybrid proteins<sup>8</sup>. The polyubiquitin (*UBI4*) gene is dispensable in growing cells but becomes essential (as the main supplier of ubiquitin) during stress<sup>8</sup>.

The tail amino-acid sequences of the ubiquitin precursors are highly conserved between yeast and mammals<sup>7,24,25</sup>, suggesting that they function similarly in all eukaryotes. The yeast *UBI1* and *UBI2* genes encode identical 52-residue tails, whereas *UBI3* encodes a 76-residue tail with little amino-acid sequence similarity to the tails of the *UBI1* and *UBI2* proteins<sup>24</sup> (Fig. 1a). Both the *UBI1/UBI2* and *UBI3* tails are highly basic however, and contain cysteine-rich, putative zinc-binding domains, suggesting that these proteins may bind nucleic acids<sup>24</sup>.

We report here that these tails are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. These and related results suggest a novel, 'chaperone' function for ubiquitin, in which its covalent association with other proteins can promote the formation of specific cellular structures.

### Deletion analysis of the *UBI1-UBI3* genes

To investigate the functions of the ubiquitin-hybrid genes, we precisely deleted the coding sequences of these genes from the *S. cerevisiae* genome (see Fig. 1 legend). Unlike the deletion of *UBI4*<sup>8</sup>, deletions of the ubiquitin-hybrid genes resulted in slow growth phenotypes, with the *ubi3* deletion having a much more severe effect than the *ubi1* or *ubi2* deletions (Fig. 1c). The phenotypes of the *ubi1* and *ubi2* single mutants can be viewed essentially as gene dosage effects, because *UBI1* and *UBI2* encode identical proteins<sup>24</sup> and because the *UBI2* gene, carried on a centromere-containing (*CEN*) plasmid, fully complemented the growth defect of the *ubi1* mutant (data not shown). The *ubi1 ubi2* double mutant is not viable, as indicated by our inability to recover *ubi1 ubi2* meiotic segregants upon sporulation of a *ubi1/+*, *ubi2/+* diploid, and by the fact that a yeast plasmid carrying the *UBI2* gene and an origin of replication, but lacking a *CEN* element was fully stabilized against mitotic loss in a *ubi1 ubi2* genetic background (data not shown). Thus, *UBI1* and *UBI2* encode an essential protein and seem to be functionally interchangeable genes.

The relative contributions of *UBI1-UBI4* to steady-state levels of free ubiquitin were assessed by immunoblotting. Deletion of any one of the four yeast ubiquitin genes resulted in, at most, a slight decrease in the level of free ubiquitin (Fig. 1c). Strikingly, there was no correlation between growth rates of the various deletion mutants and their levels of free ubiquitin (Fig. 1c). The growth defects of the *ubi1*, *ubi2* and *ubi3* deletion mutants must therefore be due, at least in part, to either the absence or decrease in concentration of specific tail components of the *UBI1-UBI3* proteins.

To further dissect the contributions of ubiquitin and the tails to the functions of the ubiquitin-hybrid genes, genetic elements encoding the tail and ubiquitin moieties were separated from each other in plasmid constructs, introduced into the mutant strains described above, and tested for their ability to complement the cognate deletion mutations (Fig. 2). Oligonucleotide-directed mutagenesis was used to construct a plasmid in which the entire ubiquitin-coding component of *UBI3*, except for its initiator ATG codon, had been deleted. This plasmid, which

retained the flanking regions of the *UBI3* gene, but expressed the *UBI3* tail in a ubiquitin-free form, fully complemented the growth deficiency of the *ubi3* deletion mutant. Other *ubi3* phenotypes, such as hypersensitivity to nitrogen starvation, defective processing of RNA precursors (see below) and the abnormally large size of *ubi3* cells, were also complemented by the *UBI3* tail-expressing plasmid. Although in these experiments the *UBI3* tail gene was carried on a low copy number *CEN* plasmid, the observed full complementation of the *ubi3* phenotype depended on a moderate amplification of the plasmid copy number (see below). In contrast to the *UBI3* tail, free ubiquitin, when expressed from the *UBI3* promoter, did not complement any of the *ubi3* phenotypes significantly. A plasmid, pUb39, which expressed free ubiquitin from the *CUP1* promoter<sup>26</sup> at a rate comparable to that of *UBI1-UBI4* combined, also failed to complement *ubi3* (Fig. 2).

In analogous experiments with *UBI1*, we expressed ubiquitin and the *UBI1* tail separately using heterologous promoters, as plasmids carrying the *UBI1* promoter sequence were unstable in yeast. Expression of the free *UBI1* tail from the strong *GPD* promoter<sup>27</sup> complemented the lethal phenotype of the *ubi1 ubi2* double deletion and supported wild-type growth rates in this genetic background (Fig. 2). These results indicated that the *UBI1* and *UBI2* tails are essential proteins and can function without an N-terminal ubiquitin moiety. In a complementary experiment, the free ubiquitin-expressing plasmid pUb39 failed to rescue the viability (ability to germinate) of *ubi1 ubi2* spores (Fig. 2).

Vector	Promoter	Product	Complementation			
			<i>ubi1 ubi2</i>	<i>ubi3</i>	<i>ubi1 ubi2 ubi3</i>	
pUb94	GPD	UBI1 tail	+	ND	ND	
pUb36	UBI3	UBI3 tail	ND	+	ND	
pUb95	GPD UBI3	UBI1 tail UBI3 tail	+	+	+	
pUb39	CUP1	Ubiquitin	—	—	—	
pUb35	UBI3	Ubiquitin	ND	—	ND	

FIG. 2 Summary of plasmid-based genetic complementation tests. Plasmids were transformed as described<sup>46</sup> into singly or multiply heterozygous diploids of the appropriate genotype. Transformants were sporulated and appropriate meiotic products of both mating types were tested for viability and growth rates. ND, not determined.

**METHODS.** Growth was assayed in media selective for the plasmid-borne selectable marker, wild-type growth being taken as that of a chromosomally wild-type strain transformed with the parental plasmid lacking ubiquitin genes and their derivatives. To construct pUb94, the ubiquitin-coding element of *UBI1* was deleted (except for its start codon) from a *UBI1*-containing DNA fragment, as described<sup>47</sup> (see also Fig. 1 legend). A 340-bp *HinfI* fragment containing the tail-coding element of *UBI1* was ligated to *Bam*H1 linkers and subcloned into *Bam*H1-digested pGPD(G)-2 (ref. 27; pGPD(G)-2 has a UAS<sup>gal</sup>-containing DNA fragment inserted into the *SalI* site of pGPD(s)-2; G. Bitter, personal communication) to yield pUb92. In this way the *GPD* promoter was positioned in place of the *UBI1* promoter. The expression cassette thus constructed was subcloned into the *SmaI* site of YE351 (ref. 49), yielding pUb94. pUb36 is a YCp50-based<sup>50</sup> vector containing a *UBI3* tail-coding element (the ubiquitin-coding element is deleted), about 700 bp of 5' noncoding sequence from the *UBI3* gene, and about 400 bp of 3' noncoding sequence. pUb95 was constructed by subcloning the pUb36 insert into the unique *XbaI* site of pUb94. pUb39 is identical to YE46 (ref. 26) except that it carries the *LYS2* rather than *TRP1* selectable marker. pUb35 is identical to pUb36 except that its insert contains the *UBI3* ubiquitin-coding element (the tail-coding element is deleted). Because the expression of the *UBI1* tail from the plasmids pUb94 and pUb95 is galactose-dependent, glucose was replaced in the medium by 2% galactose, 2% glycerol, 2% ethanol and 40  $\mu\text{g ml}^{-1}$  aspartic acid<sup>51</sup> when these plasmids were assayed for complementation.

These results indicate that the tails of ubiquitin-hybrid proteins have growth-related functions and that location of the tails within ubiquitin-hybrid proteins is not strictly essential for tail function. It has been proposed that the ubiquitin-tail fusion arrangement may instead be required for ubiquitin function<sup>28</sup>; the tails, by virtue of their apparent nuclear targeting signals<sup>24,28</sup>, might mediate transport of ubiquitin to the nucleus. To test this possibility, a strain entirely lacking ubiquitin-hybrid proteins was generated. We constructed a plasmid, pUb95, that expressed both *UBI1* and *UBI3* tails in a ubiquitin-free form and transformed it into a *ubi1/+*, *ubi2/+*, *ubi3/+* diploid, which was sporulated and subjected to tetrad analysis (Fig. 2). In the presence of the free tail-expressing plasmid, *ubi1 ubi2 ubi3* meiotic products could be recovered as viable cells, indicating that linkage of the tails to ubiquitin within the ubiquitin-hybrid proteins does not support an essential function of ubiquitin. The polyubiquitin gene, *UBI4*, the only ubiquitin gene that remained in the *ubi1 ubi2 ubi3* mutants, was strongly induced in this genetic background (unpublished data).

### Deficiency of ribosomal subunits

In the course of a northern hybridization experiment, RNA preparations from the various *ubi1-ubi3* mutants were analysed electrophoretically. This revealed a strikingly substoichiometric ratio of 18S to 25S rRNA in the *ubi3* mutants (Fig. 3a). The 18S and 25S rRNAs reside within the small (40S) and large (60S) ribosomal subunits, respectively, and their normal 1:1 stoichiometry is maintained partly because they are derived from a single precursor transcript<sup>29,30</sup>.

To test whether the deficiency of 18S rRNA in the *ubi3* mutants was accompanied by a deficiency of small ribosomal subunits, we prepared extracts from *ubi3* and wild-type cells under conditions which dissociate translating polyribosomes<sup>31</sup>. The extracts were sedimented through 15–30% sucrose gradients at low ionic strength, which promotes the association of large and small ribosomal subunits into 80S ribosomes (monosomes). The main sedimentation peak in the wild-type extract was monosomal (Fig. 4a). In contrast, the main peak in the *ubi3* extract was composed of free 60S subunits, and the monosome peak was greatly reduced. These results indicate that the *ubi3* mutants contain substoichiometric quantities of small ribosomal subunits.

To determine whether the *ubi3* mutant was defective in the formation (rather than metabolic stability) of 40S ribosomal subunits, we examined the processing of pre-rRNA in mutant and wild-type cells. Pre-rRNA processing was monitored using a pulse-chase procedure, followed by agarose gel electrophoresis and fluorography. The results are shown in Fig. 3c and d, together with a diagram of the pre-rRNA processing pathway (Fig. 3b). After a 6-min pulse, the predominantly labelled species are the 27S and 20S RNAs, which are the immediate precursors of the mature 25S and 18S rRNAs, respectively. In wild-type cells at 30 °C, these intermediate species are processed to completion within 30 min, whereas in the *ubi3* cells the rate at which the 18S rRNA is generated from the 20S pre-rRNA is dramatically reduced, and the bulk of the 20S pre-rRNA is ultimately degraded rather than processed. In contrast, the conversion of the 27S precursor to the 25S rRNA (residing in the large ribosomal subunit) is normal in the *ubi3* mutant (Fig. 3d). The 20S pre-rRNA processing defect cannot be accounted for by a defect in its transport to the cytoplasm (data not shown), where the 20S pre-rRNA is normally converted to 18S rRNA<sup>29</sup>. Thus, a specific pre-rRNA processing defect explains the aberrant stoichiometry of rRNA in the *ubi3* mutant and accounts, at least in part, for its growth defect.

Remarkably, both the *ubi1 ubi3* and *ubi2 ubi3* double mutants grow faster than the *ubi3* single mutant, despite their relatively low free ubiquitin levels (Fig. 1c). This is particularly striking as the *ubi1* and *ubi2* single mutants grow more slowly than wild-type cells (Fig. 1c). Thus, *ubi1* and *ubi2* are both weak

suppressors of *ubi3*. Figure 3a shows that *ubi1* and *ubi2* also partially restore the stoichiometry of 18S rRNA to 25S rRNA in the *ubi3* genetic background. This result indicates that *UBI1* and *UBI2* are, like *UBI3*, involved in some aspect of ribosome assembly or turnover. Although the ratio of 18S rRNA to 25S rRNA, as estimated from gel electrophoretic patterns (Fig. 3a), is not noticeably abnormal in either *ubi1* and *ubi2* mutants, a more sensitive assay, namely sucrose gradient sedimentation, revealed a deficiency of 60S subunits in the *ubi1* mutant, in contrast to the *ubi3* mutant which was deficient in 40S subunits (Fig. 4b). A slight reduction in the monosome level (relative to total RNA in the extract) was also apparent in the *ubi1* cells (Fig. 4b; compare the 80S peak to the peak at the top of the gradient). Although the 60S subunit deficiency of *ubi1* cells is moderate, it is presumably sufficient to account for the mild

growth defect of these cells (Fig. 1c), which still express a protein identical to *UBI1* from the *UBI2* gene.

The partial suppression of the *ubi3* growth defect by the *ubi1* and *ubi2* mutations (Fig. 1c) may result from their lowering of 60S subunit levels and thus partially alleviating the relative deficiency of 40S subunits, perhaps without significantly affecting the absolute levels of 40S subunits. Although the coupling of 40S and 60S subunits within the ribosome only occurs after the 40S subunit associates with mRNA, the two subunits have significant residual affinity for each other in the absence of mRNA<sup>32</sup>. As a result, the abnormally high level of free 60S subunits in the *ubi3* mutant (Fig. 4a) may lead to partial sequestration of 40S subunits, thereby further decreasing the concentration of free 40S subunits available for translation in *ubi3* cells. This effect, analogous to the 'squelching' effect described for transcriptional regulators<sup>33</sup>, should become weaker after a decrease in the level of 60S subunits, possibly accounting for the suppressive effect of the *ubi1* and *ubi2* mutations in the *ubi3* genetic background (Figs 1c and 3). As the growth of the *ubi3* mutant is cold-sensitive (relative to wild-type), whereas the ratio of 18S rRNA to 25S rRNA in *ubi3* cells does not change at low temperatures (data not shown), the hypothetical sequestration effect may decrease at higher temperatures. Alternatively, those few 40S ribosomal subunits which do form in the absence of the *UBI3* tail may be intrinsically

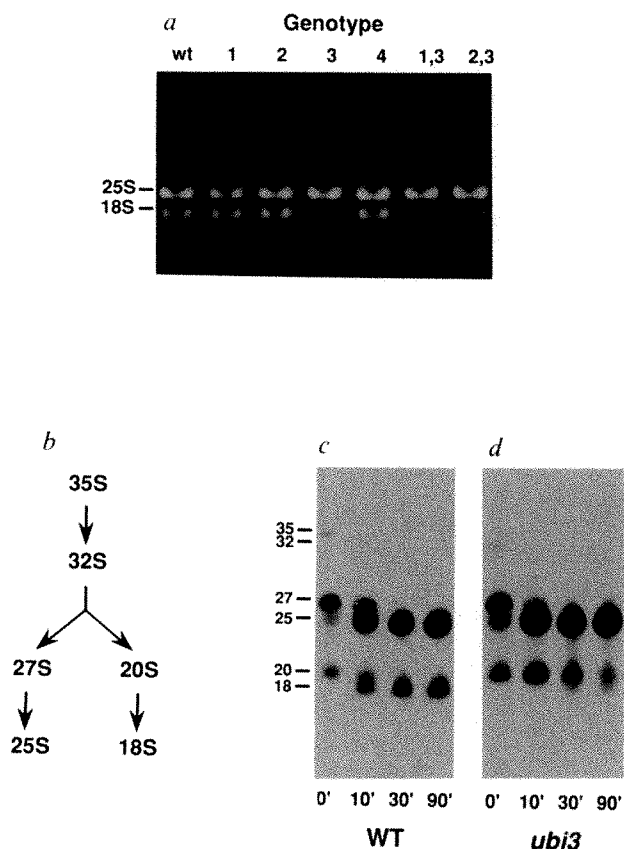


FIG. 3 Defective pre-rRNA processing and reduced levels of 18S rRNA in the *ubi3* mutant. *a*, RNA was prepared from mutant and wild-type cells grown in YPD, electrophoresed on a 1% agarose gel in the presence of ethidium bromide and photographed under ultraviolet light. Lanes are numbered according to the ubiquitin gene or genes deleted in the corresponding strain. *b*, Schematic representation of the pathway of pre-rRNA processing in *S. cerevisiae*. 35S RNA is the primary transcript. *c*, *d*, Pulse-chase analysis of pre-rRNA processing in wild-type and *ubi3* deletion strains. rRNA precursors in exponentially growing yeast cells were labelled (in minimal medium supplemented with auxotrophic nutrients<sup>46</sup>) with [methyl-<sup>3</sup>H]methionine (Amersham, 80 Ci mmol<sup>-1</sup>) for 6 min at 23 °C. ([methyl-<sup>3</sup>H] methionine donates methyl groups to newly synthesized pre-rRNA<sup>29</sup> via the general methyl donor *S*-adenosylmethionine.) Unlabelled methionine (1 mg ml<sup>-1</sup>) was added to initiate the chase incubation, and RNA was prepared from the cells at the times indicated. RNA was electrophoresed on a 1% agarose gel and visualized by fluorography. Molecular weights are shown on the left, in thousands. METHODS. RNA was prepared from exponentially growing cells as described previously<sup>52</sup>, except that 0.2% diethylpyrocarbonate was added to the lysis buffer and SDS was omitted. RNA (10 µg per lane) was electrophoresed on formaldehyde-containing gels as described previously<sup>47</sup>, except that formaldehyde was used at 0.2 M, and iodoacetamide (10 mM) and ethidium bromide (0.5 µg ml<sup>-1</sup>) were also added to the gel (B. Seed, personal communication). All strains analysed were *MATα*.

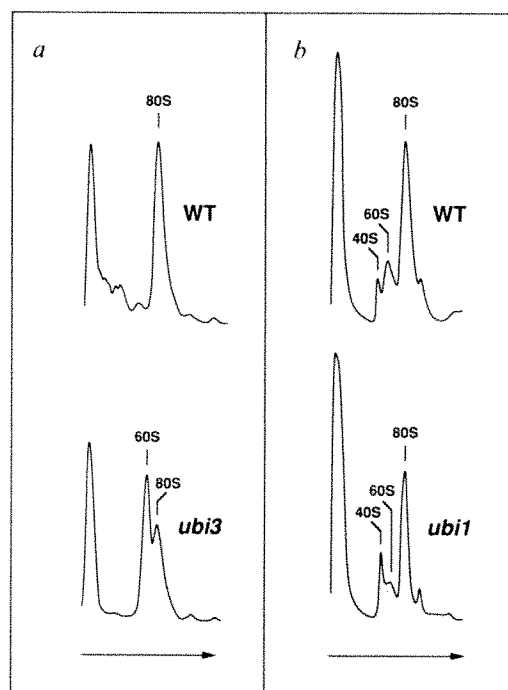


FIG. 4 Aberrant stoichiometries of ribosomal subunits in *ubi1* and *ubi3* mutants. Sedimentation analysis of subunits present in cytoplasmic extracts from spheroplasts of either (a) wild-type and *ubi3* strains, or (b) wild-type and *ubi1* strains. Direction of sedimentation is indicated by an arrow. METHODS. Spheroplasts were prepared from cells grown in YPD by digestion with zymolyase as described previously<sup>31</sup>. After allowing the spheroplasts to recover<sup>31</sup>, sodium azide was added, and the culture was brought to 8 °C for 5 min to induce polyribosome 'run off'<sup>31</sup>. Spheroplasts were then lysed as described<sup>31</sup>, except that heparin (0.2 mg ml<sup>-1</sup>) was added to the lysis buffer<sup>53</sup>. Extracts were centrifuged at 20,000 *g* for 15 min. In *b*, the ionic strength of the extract was then reduced by diluting it threefold into gradient buffer. Supernatants were loaded onto 15–30% linear sucrose gradients prepared to 50 mM Tris-HCl (pH 7.4), 50 mM KCl, 5 mM MgCl<sub>2</sub>, 2 mM dithiothreitol and centrifuged at either 39,000 r.p.m. for 3 h (a) or 35,000 r.p.m. for 5 h (b) in an SW41 rotor (Beckman) at 5 °C. Gradients were fractionated by upward displacement in an ISCO gradient fractionator equipped with a UV monitor. Peak assignments are based on electrophoretic analysis of RNA prepared from appropriate gradient fractions. All strains analysed were *MATα*.



cold-sensitive, presumably because they lack the UBI3 tail (see below).

### The tails are ribosomal proteins

The above results, which indicate that the UBI3 tail participates in ribosome biogenesis, suggested that this tail was either a component of the pre-rRNA processing apparatus, which seems to include a variety of small nuclear ribonucleoprotein particles<sup>34</sup>, or a ribosomal protein whose incorporation into the nascent ribosome facilitates recognition of the 20S pre-rRNA by specific processing factors. To distinguish between these possibilities, we added an immunological marker to the C-terminus of the UBI3 protein by fusing the *UBI3* gene with a sequence encoding a 12-residue epitope from the human *c-myc* gene product<sup>35</sup>. The resulting *UBI3-myc* gene fully complemented the growth defect of the chromosomal *ubi3* deletion, indicating that the C-terminal Myc peptide did not obstruct the activity of the UBI3 protein. Whole-cell extracts of wild-type (*UBI3*) cells and of *UBI3-myc* cells carrying a chromosomal deletion of *ubi3* were fractionated by SDS-PAGE, transferred onto filters and probed with a monoclonal antibody to the Myc peptide. A single band was observed in immunoblots of *UBI3-myc* cells, whereas extracts from wild-type cells were essentially free of antibody-binding species (Fig. 5a, lane W). The immunoreactive protein is apparently the deubiquitinated form of UBI3-Myc, as it is not recognized by antibodies to ubiquitin, and its apparent molecular weight is considerably less than that of UBI3-Myc produced in *Escherichia coli* (Fig. 5a). This, with the data of Fig. 2, suggests that the UBI3 hybrid protein is efficiently processed (deubiquitinated) *in vivo*. Rapid *in vivo* deubiquitination in eukaryotic cells (but not in bacteria) is also characteristic of engineered ubiquitin fusions such as ubiquitin- $\beta$ -galactosidase<sup>36</sup>.

Extracts from wild-type and *UBI3-myc* cells were analysed on sucrose gradients under optimal conditions for the resolution of polyribosomes (Fig. 5a). The proteins of the monosome and polyribosome peaks were fractionated by SDS-PAGE, transferred onto filters and probed with the anti-Myc antibody. The Myc-tagged UBI3 tail protein was found primarily within rapidly sedimenting structures, and co-sedimented with both 80S monosomes and the broadly distributed polyribosomes (Fig. 5a). A second extract was prepared from *UBI3-myc* cells under conditions that dissociate polyribosomes, and centrifuged in the presence of a high ionic strength buffer, which dissociates monosomes into free subunits. Under these conditions the UBI3 tail co-sedimented with the 40S ribosomal subunit (Fig. 5b). Taken together, these data indicate that the UBI3 tail is a component of the small subunit of the ribosome. The UBI3 tail does not seem to be a processing or assembly factor that is transiently associated with nascent 40S subunits because it is also present within translationally active ribosomes. The association of the UBI3 tail with the 40S subunit persists under high salt conditions (0.5 M KCl), suggesting that the UBI3 tail is tightly associated with mature ribosomes.

To localize the UBI1 tail protein, we again used the Myc tagging method. A *UBI1 tail-myc* gene in which the Myc sequence was positioned upstream of the tail-coding sequence in place of the ubiquitin-coding sequence, rescued the viability of *ubi1 ubi2* mutants and restored their growth rates to near wild-type levels. Extracts from *UBI1 tail-myc* cells were fractionated on sucrose gradients optimized for the analysis of either polyribosomes or ribosomal subunits, as described above for *UBI3-myc*. On the polyribosome gradient, the UBI1 tail co-sedimented with 80S monosomes and polyribosomes (Fig. 5c), whereas on the ribosomal subunit gradient the UBI1 tail co-sedimented with the 60S subunit (Fig. 5d). Thus, like the UBI3 tail, the UBI1 tail is tightly associated with mature, translationally active ribosomes. We conclude that the UBI3 tail, and the identical UBI1 and UBI2 tails, are ribosomal proteins which reside in the small and large subunits, respectively. The main

structural motifs of the UBI1-UBI3 proteins, their putative zinc fingers<sup>24</sup>, are presumably involved in binding rRNA.

Although both UBI1 tail-Myc and UBI3-Myc were found in ribosomes, their localization to distinct ribosomal subunits precludes the possibility that these associations were mediated artefactually by the Myc peptide. Moreover, the human homologue of the UBI3 tail protein has independently been localized to the small ribosomal subunit<sup>37</sup>. Our data do not exclude the possibility that a fraction of the UBI1 and UBI2 tails are present within yeast ribosomes in an unprocessed, ubiquitin-containing form. The presence of significant levels of free ubiquitin in *ubi3 ubi4* mutants (Fig. 1c) does indicate, however, that the UBI1 and UBI2 gene products can be deubiquitinated; furthermore, the full complementing activity of the free UBI1 tail indicates that ribosomes containing the free UBI1 tail are functionally indistinguishable from wild-type.

Examination of the previously determined<sup>24</sup> nucleotide sequences upstream of the *UBI1*, *UBI2* and *UBI3* genes revealed near-matches to the consensus sequence<sup>30</sup> of upstream activation sites (UAS) within genes for ribosomal proteins (Fig. 1b). (Such UAS<sup>TP8</sup> sites have also been found within a few genes encoding non-ribosomal proteins<sup>30</sup>.) The *UBI1*, *UBI2* and *UBI3* promoter regions also contain T-rich stretches which represent a second sequence motif characteristic of promoters of ribosomal protein genes<sup>30</sup> (Fig. 1b). In *UBI1-UBI3*, the positioning of these UAS and T-rich sequences with respect to each other and to sites of translational initiation, is similar to that of known ribosomal protein genes and consistent with the proposed function of these sequences in transcriptional regulation. With the exception of genes for ribosomal proteins, few yeast genes contain introns; their presence within the *UBI1* and *UBI2* genes (Fig. 1a) further indicates that the sequence features of *UBI1-UBI3* are typical of ribosomal protein genes.

### Ubiquitin and ribosome biogenesis

The tandem arrangement of ubiquitin and specific ribosomal proteins (tails) within proteolytically processed precursors has been conserved throughout the evolution of eukaryotes<sup>7,25</sup>. Nevertheless, expression of the tails in a ubiquitin-free form can fully complement chromosomal mutations in which the corresponding ubiquitin-tail gene is entirely deleted (see above). To test further for a function of the cotranslational synthesis of ubiquitin and the tail proteins, we integrated the free tail-expressing derivative of the *ubi3* gene (*ubi3 $\Delta$ ub*) into its natural chromosomal locus. The complete deletion mutant, *ubi3*, which grows slowly (Fig. 1c), was transformed with linear DNA fragments carrying either the ubiquitin deletion mutation *ubi3 $\Delta$ ub* or the wild-type *UBI3* gene and growth revertants were selected. Growth revertants resulting from transformation with *ubi3 $\Delta$ ub* DNA were predominantly of two classes: those growing at wild-type rates (1.6-h doubling time) and those in which the *ubi3* growth defect had been only partially reverted (3-h doubling time, compared with the 6.8-h doubling time of the *ubi3* mutant). Southern hybridization analysis (data not shown) indicated that in all seven of the revertants with wild-type growth rates, the *ubi3* mutation had been replaced by multiple tandem insertions of *ubi3 $\Delta$ ub* DNA, whereas in all 12 partial growth revertants analysed, *ubi3* had been replaced with a single, properly integrated *ubi3 $\Delta$ ub* sequence (see Fig. 6a for a comparison of growth rates; we refer to the integrated, single-copy *ubi3 $\Delta$ ub* allele as *ubi3 $\Delta$ ub-1*, and to the integrated multiple-copy allele taken for analysis as *ubi3 $\Delta$ ub-2*). In contrast, single-copy integration of wild-type (*UBI3*) DNA resulted in complete reversion of the *ubi3* growth defect (Fig. 6a). These results, which strongly suggested that the *ubi3 $\Delta$ ub* allele can fully complement *ubi3* only when present in multiple copies, were confirmed by showing that in a *ubi3* genetic background, the copy number of CEN plasmids carrying the *ubi3 $\Delta$ ub* allele was several times higher than that of CEN plasmids carrying the *UBI3* allele, whereas in a wild-type genetic background such

plasmids had equivalent copy numbers (data not shown). Thus, regardless of whether the free tail-expressing (*ubi3 $\Delta$ ub*) allele is carried on a *CEN* plasmid or integrated into the chromosome, full complementation of the *ubi3* phenotype requires approximately three to fourfold amplification of the *ubi3 $\Delta$ ub* copy number.

To determine the stoichiometry of 18S to 25S rRNA in the *ubi3 $\Delta$ ub-1* mutant (which contained the integrated, single-copy *ubi3 $\Delta$ ub* allele), RNA from this and control strains was electrophoresed on an agarose gel and visualized by ethidium staining (Fig. 6b). A deficiency of 18S rRNA was clearly visible in the *ubi3 $\Delta$ ub-1* mutant, although it was not as pronounced as in the *ubi3* mutant (Fig. 6b). A wild-type (1:1) stoichiometry of 18S rRNA to 25S rRNA was seen in the *ubi3 $\Delta$ ub-2* mutant, which contained multiple copies of *ubi3 $\Delta$ ub* (Fig. 6b). Thus, deletion of the ubiquitin-coding element of *UBI3* leads to a deficiency of 18S rRNA which can be suppressed by amplifying the copy number of the free tail-expressing allele of the *UBI3* gene. This indicates that the ubiquitin-coding portion of the *UBI3* gene, although not strictly required for the processing of

20S pre-rRNA, facilitates this processing reaction, apparently by increasing the efficiency of incorporation of the *UBI3* tail into the nascent 40S ribosomal subunit.

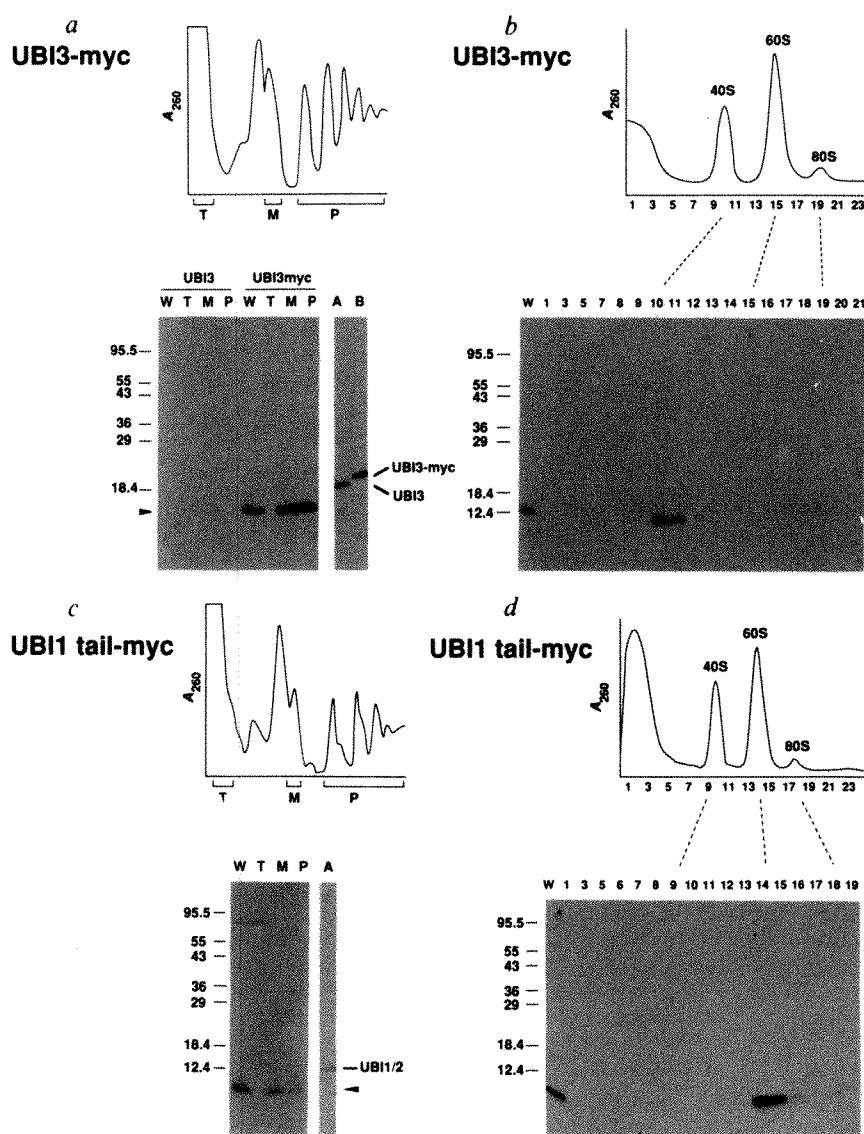
To test whether the effect of deleting the ubiquitin coding sequence of *UBI3* on tail function was achieved through a post-translational mechanism, *UBI3* mRNA levels were determined in *ubi3 $\Delta$ ub-1* and control strains. The northern hybridization analysis of Fig. 6c showed that the effect of the *ubi3 $\Delta$ ub-1* mutation on *UBI3* tail function is not achieved by decreasing the level of *UBI3* mRNA; instead, the *ubi3 $\Delta$ ub-1* mRNA is considerably more abundant than that of *UBI3*. Thus, the primary functional defect of the *ubi3 $\Delta$ ub-1* mutant lies in either a cotranslational or post-translational process. We conclude that the effective levels of newly synthesized *UBI3* tail protein, as reflected by its capacity to accelerate the processing of 20S pre-rRNA (Figs 3, 6), are significantly enhanced by its transient covalent association with ubiquitin.

## Discussion

Ubiquitin has previously been shown to function as a post-

FIG. 5 Immunoblot analysis of *S. cerevisiae* extracts fractionated in sucrose gradients to resolve either polyribosomes (a, c) or dissociated 40S and 60S subunits (b, d). In panels a and c, fractions were pooled as shown: W, whole-cell extract; T, top of gradient; M, monosomes; P, polyribosomes. a, Polyribosomal fractions from *ubi3* cells carrying either *UBI3* (lanes 1–4) or *UBI3-myc* (lanes 5–8) on a *CEN* plasmid, probed with anti-Myc antibody. Lanes A and B show the unprocessed (non-deubiquitinated) *UBI3* and *UBI3-myc* produced in *E. coli* and probed with anti-ubiquitin antibody<sup>21</sup>. b, Ribosomal subunit fractions from *ubi3* cells carrying *UBI3-myc* on a *CEN* plasmid, probed with anti-Myc antibody. c, Polyribosomal fractions from *ubi1 ubi2* cells carrying *UBI1 tail-myc* on a 2 $\mu$  plasmid, probed with anti-Myc antibody. Lane A shows unprocessed *UBI1* produced in *E. coli* and probed with anti-ubiquitin antibody. d, Ribosomal subunit fractions from *ubi1 ubi2* cells carrying *UBI1 tail-myc* on a 2 $\mu$  plasmid, probed with anti-Myc antibody. In c, the monosome and polyribosome peaks each have a shoulder of more rapidly sedimenting material. Similar results have been obtained with unrelated mutants deficient in large ribosomal subunits<sup>54</sup>, as is the *ubi1* strain (Fig. 4). The shoulder seems to represent mRNAs which have bound the 40S ribosomal subunit to form a preinitiation complex, but have not proceeded to bind a 60S subunit because the level of free 60S subunits is low<sup>54</sup>. Molecular weights are shown in thousands.

**METHODS.** To construct pUb54, the 1.3-kb *HincII* fragment from *UBI3* was ligated to the 0.7-kb *HincII*–*SphI* fragment of the plasmid AHSM (ref. 55) and inserted into YCp50 (ref. 50). The resulting plasmid encodes *UBI3-myc*, in which the last two amino acids of *UBI3* are replaced by the 12-residue Myc tag. To construct pUb62, the ~30-bp *Bam*HI–*Hind*III fragment of pUb92, encoding the first seven residues of the *UBI1* tail, was replaced by a synthetic oligonucleotide encoding the 12-residue Myc tag followed by the first seven residues of the *UBI1* tail. (A C-terminal fusion of Myc to *UBI2* did not complement the *ubi1 ubi2* double mutation; data not shown). The resulting plasmid was cut with *Eco*RI and *Eco*RV and the 2.3-kb fragment containing *UBI1 tail-myc* was cloned into the *Sma*I site of YEp351 (ref. 49), yielding pUb62. For the preparation of polyribosomes, cells were grown to an  $A_{600}$  ~0.5. Extracts were prepared<sup>31</sup> and analysed<sup>53</sup> as described. Gradients were centrifuged in an SW41 rotor at 15,000 r.p.m. for 16 h. Proteins were precipitated with 25% trichloroacetic acid, the pellets washed, dissolved and electrophoresed and electroblotted as in Fig. 1. Filters were probed with the Myc1-9E10 antibody<sup>35</sup> followed by <sup>125</sup>I-labelled sheep



anti-mouse IgG, using standard techniques<sup>8</sup>. Preparation of extracts for the analysis of ribosomal subunits was as in the Fig. 4 legend, except that extracts were brought to 0.5 M KCl before loading onto 15–35% sucrose gradients prepared as in the Fig. 4 legend but with the addition of 0.5 M KCl. Gradients were centrifuged in an SW41 rotor at 21,000 r.p.m. for 17 h at 10 °C.

translational modifying group which signals the degradation of acceptor proteins. Here, we describe a second mechanism of ubiquitin function in which ubiquitin is cotranslationally joined to an acceptor protein and serves to promote the assembly of the acceptor protein into a specific cellular structure. The role we have described for ubiquitin in the biogenesis of ribosomes in yeast probably applies to eukaryotes in general, as close homologues of the *UBI1-UBI3* genes exist in animals, plants

and lower eukaryotes<sup>7,25</sup>. Moreover, sequences homologous to the tail components of *UBI1-UBI3* have invariably been found fused to ubiquitin.

*UBI1-UBI3* form a subset of a class of genes in which an element encoding either ubiquitin or an amino-acid sequence similar to that of ubiquitin is fused to a downstream coding sequence. Although no systematic effort to identify such genes has yet been reported, four loci encoding C-terminally extended ubiquitin-like polypeptides have already been described. These are: first, the *An-1* gene, whose transcripts are segregated to the animal pole of *Xenopus laevis* embryos (D. Weeks and D. Melton, personal communication); second, a human gene that encodes an interferon-inducible protein with a relative molecular mass of about 15,000 (refs 38, 39); third, the constitutively expressed human gene *GdX* (ref. 40); and finally, the large open reading frame of the togavirus BVDV (bovine viral diarrhoea virus), which encodes a ubiquitin-like sequence embedded within the nonstructural viral polyprotein<sup>41</sup>. Because of their substantial divergence from the canonical, highly conserved ubiquitin sequence, the ubiquitin-like proteins cannot serve as precursors to ubiquitin, in contrast to the *UBI1-UBI3* proteins. Moreover, the processing proteases that remove ubiquitin from ubiquitin fusion proteins are expected to be virtually inactive upon the ubiquitin-like proteins, because the bond that would be cleaved is flanked by amino-acid substitutions that block (or are expected to block) cleavage of engineered ubiquitin fusion proteins<sup>36,42</sup>. We suggest that the function of ubiquitin-like protein domains may be to enhance the function of linked non-ubiquitin domains in a way similar to that seen in the *UBI3* protein.

It has recently been shown that certain proteins, known as 'molecular chaperones', associate transiently (and non-covalently) with newly synthesized polypeptide chains and so facilitate their incorporation into oligomeric structures<sup>43-45</sup>. By definition, molecular chaperones do not form part of the final oligomeric structure, nor do they necessarily possess steric information specifying the structure's assembly<sup>43</sup>. By these criteria, it seems that the ubiquitin moiety of the *UBI3* protein acts as a molecular chaperone of the *UBI3* tail in facilitating its incorporation into ribosomes. The covalent, cotranslational association of a chaperone protein with its ligand has several potential advantages: the N-terminal chaperone is bound to and can protect its ligand from the moment the ligand is synthesized; the chaperone can transiently block, or shelter, the N-terminus of its ligand (which may serve as a signal for degradation in some proteins<sup>36</sup>); and finally, the cotranslational chaperone can bind its ligand without needing a specific recognition site in the ligand.

It is probable that the ubiquitin tails, like other ribosomal proteins<sup>30</sup>, are extremely short-lived if they are not assembled within ribosomes. Thus, the transient attachment of ubiquitin to the N-termini of the *UBI1-UBI3* tails could increase the efficiency of their incorporation into nascent ribosomes by a transient metabolic stabilization of the newly formed tails. Although ubiquitination of a protein can signal its degradation, this activity seems to require multi-ubiquitin chains<sup>23</sup>. Rather than directly protecting the *UBI1-UBI3* tails against degradation, ubiquitination could increase the rate of the tails' transport to, or assembly within, nascent ribosomes, thus decreasing the proportion of newly synthesized tails that are degraded in transit to the ribosome. Experiments to distinguish between these possibilities are in progress. □

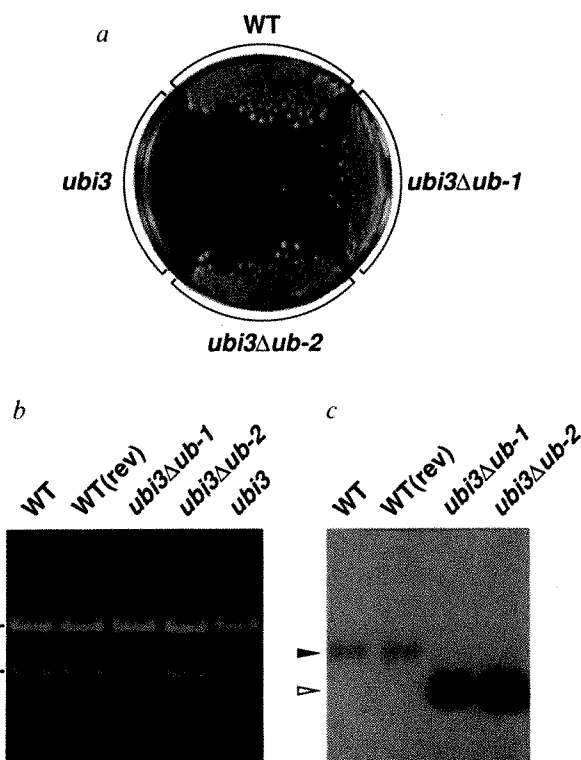


FIG. 6 Characterization of mutants bearing single or multiple copies of the *ubi3Δub* allele. *a*, Growth defect of the *ubi3Δub-1* mutant. Cultures were streaked onto YPD plates and incubated for 3 days at 30 °C. Quadrant designations are as follows: WT, wild-type; *ubi3Δub-1*, a growth revertant obtained through integrative transformation of a *ubi3* strain with a DNA fragment carrying the *ubi3Δub* deletion mutation (single-copy integration); *ubi3Δub-2*, as *ubi3Δub-1*, except that 3–4 copies of the transforming DNA fragment integrated tandemly at the *UBI3* locus; *ubi3*, which forms sizeable colonies only after 1 week. *b*, 18S rRNA deficiency in the *ubi3Δub-1* mutant. RNA was resolved on a 1% agarose gel in the presence of ethidium bromide, and photographed under UV light. Designations are as in *a*, except for lane WT (rev), which contained RNA from a phenotypically wild-type revertant obtained through integrative transformation of a *ubi3* strain with a DNA fragment carrying the wild-type *UBI3* gene. *c*, Northern hybridization analysis of *UBI3* RNA levels in mutant strains. Closed arrow, *UBI3* RNA; open arrow, *ubi3Δub* RNA.

**METHODS.** Growth revertants were selected on YPD plates at 23 °C, a temperature at which the (cold-sensitive) *ubi3* mutants form colonies only after more than two weeks. Transformants were screened for loss of the *ubi3*-linked *HIS3* marker before Southern hybridization analysis. *b* and *c*, RNA was prepared from cells grown in YPD, and electrophoresed as in Fig. 3, except that in *c* a 1.5% agarose gel was used, then transferred onto a Gene Screen filter (New England Nuclear), and UV-irradiated as described<sup>8</sup>. Hybridization was carried out at 37 °C in a solution containing 40% formamide, 7% SDS, and 5×SSPE. The filter was washed at 62 °C in 1% SDS, 1×SSPE. The *UBI3* hybridization probe was the insert from pUB36 (see the Fig. 2 legend). The probe was radiolabelled as described<sup>8</sup>. In *c*, 10 μg of RNA was loaded onto each lane; *b*, loads were slightly adjusted to equalize the intensities of the 25S rRNA bands to facilitate comparison of 25S rRNA:18S rRNA ratios between the lanes. To increase the sensitivity of detection, formaldehyde was omitted from the gel in *b*. In *c*, only one *UBI3* mRNA (of ~0.7 kb) was detectable in wild-type cells. This and other data, including an analysis of transcripts in the *ubi3* mutant (data not shown), indicate that the 1.8 kb and 1.0 kb mRNAs previously considered to be additional *UBI3* transcripts<sup>24</sup> are not in fact derived from the *UBI3* locus.

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## LETTERS TO NATURE

## An alternative binary model for SN1987A

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FABIAN *et al.*<sup>1</sup> and Joss *et al.*<sup>2</sup> have proposed that the progenitor of supernova 1987A was a relatively faint binary companion of the blue supergiant Sk-69°202 (ref. 3). Here we propose another class of models for SN1987A, wherein Sk-69°202 was the progenitor, but where the presupernova evolution of this star was strongly influenced by the accretion of matter from a companion star which was initially the more massive star. Binary stellar evolution calculations show that a model of this type can naturally explain why the apparent progenitor was a blue supergiant rather than a red one. The system becomes unbound after the supernova event, and the presence of a normal or neutron-star companion will probably be revealed a few years after the explosion. A model of this type can explain all the major observational features of SN1987A and also provides plausible explanations for many of its anomalies.

SN1987A has enriched many fields of astrophysics as well as some related disciplines. Surprisingly, however, the greatest challenge it has posed is to stellar evolution theory: why was the apparent progenitor (Sk-69°202; ref. 3) a blue supergiant rather than a red supergiant, the generally preferred precursor of type II supernovae<sup>4,5</sup>? Numerous single-star models for the progenitor have been advanced to explain this unexpected behaviour, but all of them face significant objections (for a review of single-star models see, for example, ref. 6 and references therein).

The resolution of this puzzle is of great importance. An understanding of the presupernova evolution would allow us to decide whether SN1987A was a unique event or was representative of a whole class of supernovae which are too faint to be easily detected outside the Local Group. Moreover, if this supernova is telling us something about the evolution of massive stars, it may turn out to be a Rosetta stone against which the theory of stellar evolution can be calibrated.

Most stars occur in binary or multiple systems<sup>7</sup>. If we allow that the supernova progenitor may have been a member of a binary, several alternative models present themselves. In one class of models, the star that exploded was not Sk-69°202, but a previously undetected companion star<sup>1,2</sup>. These models predict that Sk-69°202 will reappear when the photosphere of the supernova has receded sufficiently. In this letter we propose a different type of model, in which the progenitor was a blue rather than a red supergiant because of its past interaction with its companion. Our calculations also shed new light on the features that single-star models must have to account for the observed properties of SN1987A.

Whether a star ends its life as a red or a blue supergiant depends on some rather subtle aspects of the physics of the stellar envelope and is not entirely amenable to heuristic explanation. However, many of the essential features of the envelope physics can be described in terms of how much luminosity can be carried in the envelope by radiative diffusion (refs 8, 9; but see also refs 10 and 11). If the luminosity of a star during the course of its evolution reaches a critical value,  $L_{\text{crit}}$ , a thermal instability triggers a runaway expansion of the envelope (ref. 8) and drives the star redward toward the Hayashi track.  $L_{\text{crit}}$  is a sensitive function of the radiative opacities in the envelope and increases with decreasing opacities, a result that accounts for the requirement of very low metallicities in most single-star models for SN1987A.  $L_{\text{crit}}$  is also very sensitive to the fractional core mass  $q_c \equiv M_c/M$ , where  $M_c$  is the mass of the hydrogen-exhausted core and  $M$  is the total mass of the star. It is well known from the theory of the evolution of massive stars that a reduction in envelope mass (for example, by mass loss through a stellar wind) and a consequent increase in  $q_c$  reduces  $L_{\text{crit}}$  and favours redward motion in the Hertzsprung-Russell diagram<sup>12</sup>. Conversely, a decrease in  $q_c$  (for example, by accretion of matter from a close-binary companion) increases  $L_{\text{crit}}$ , and the transition to the red-supergiant stage may thereby be prevented (or reversed if it has already occurred).

To determine whether this mechanism may explain the seemingly anomalous colour of Sk-69°202, we have performed a series of stellar evolution calculations in which such accretion is allowed to occur. We used a standard Henyey-type code<sup>13</sup>, and we assumed an initial metallicity of  $Z = 0.02$  and that convection was governed by the Schwarzschild instability

criterion, with a convective mixing length equal to 1.5 pressure scale heights, and with the effects of convective overshooting and semiconvection included according to the prescription given by Iben<sup>14</sup>. We followed the evolution of three stellar models with initial masses of 15, 17 and 20  $M_{\odot}$  from the zero-age main sequence to the onset of core carbon burning. After the termination of the core hydrogen-burning phase, we accreted 5  $M_{\odot}$  onto the 15- $M_{\odot}$  model and 3  $M_{\odot}$  onto the 17- $M_{\odot}$  model. Thus, all three models had a final mass of 20  $M_{\odot}$ ; the masses of the hydrogen-exhausted cores were 4.5, 5.3 and 6.4  $M_{\odot}$  respectively. We assume that the time that has elapsed since the end of the mass-transfer episode is long compared with the Kelvin time,  $\tau_K \approx 10^4$  yr, of the progenitor, so that the appearance of the progenitor just before the supernova event should be essentially independent of the details of mass transfer.

The results of our calculations are illustrated in Fig. 1. Models with final values of  $q_c$  larger than a certain critical value  $q_c^0$  are red supergiants (on the Hayashi track in the H-R diagram) just before becoming supernovae, whereas models with  $q_c < q_c^0$  end their lives as blue supergiants. (For our chosen values of  $Z$  and final stellar mass, we find that  $q_c^0 \approx 0.28$ , but this value is sensitive to the parameters of the model.) The effective temperature of the star just before the supernova event increases monotonically (that is, the pre-supernova is increasingly blue) with increasing accreted mass.

Some of our detailed numerical results are sensitive to the assumed parameters of our models, but our qualitative con-

clusions regarding the viability of our binary models for SN1987A are insensitive to the assumed metallicity, the treatment of convection, the details of the mass-transfer process, and the amount of mass that may be lost by the supernova progenitor in a stellar wind following the mass-transfer phase. For our basic model to work, we require only that at least several solar masses more be accreted by the progenitor than are subsequently lost as a stellar wind. We also find that the dependence of the stellar luminosity on the core mass of the progenitor is much weaker than predicted by the core mass-luminosity relation for single massive stars in this evolutionary phase (see for example refs 15 and 16). We thus conclude that the luminosity does not accurately determine the core mass of a star that has accreted a substantial amount of matter after the termination of core hydrogen burning.

For the requisite binary evolution to occur, the supernova progenitor must have been originally the less massive star of a close binary system, because the original primary fills its critical lobe first and becomes the mass donor. From our evolutionary calculations, we also find that if mass transfer occurs before the supernova progenitor has completed core hydrogen burning, the progenitor will be rejuvenated as a main-sequence star and will subsequently mimic the standard evolution of a more massive single star; evolutionary scenarios are therefore also constrained by the requirement that the progenitor must complete core hydrogen burning before mass transfer begins. There are then two possible scenarios, involving case B (ref. 17) and case C (ref. 18) mass transfer, respectively, which have very different consequences.

In case B, the primary fills its critical lobe when it is on the first red-giant branch. As the supernova progenitor must have already left the main sequence before mass transfer begins, and as the main-sequence lifetime of a massive star is long compared with the interval from the termination of core hydrogen burning to the tip of the red-giant branch, then the original mass of the supernova progenitor must be very close to that of the primary. Using the results for our 15- $M_{\odot}$  model, we find that the original mass of the supernova progenitor must be within  $\sim 0.3\%$  of the original mass of the primary. Binary systems composed of stars with the requisite masses and initial orbital separations (of the order of a few AU) will be rather rare, although a large fraction of all close binaries do in fact contain stars of approximately equal mass<sup>7,19</sup>.

Because of the mass increase, the post-main-sequence evolution of the supernova progenitor is faster than the corresponding evolution of the original primary, and the progenitor will reach the supernova stage earlier than the primary (by as much as  $\sim 10^5$  yr for our 15- $M_{\odot}$  model). In this case, Sk - 69°202 would have had a stellar companion before the supernova event. However, as more than half the mass of the binary system should have been ejected in the supernova explosion, the system should not have remained bound, and the erstwhile companion should be revealed when or shortly after it crosses the photosphere of the supernova (unless the motion happens to be nearly directly away from the Earth). We estimate that this should occur about 1-3 yr after the explosion.

The constraint on the relative masses of the binary components for case C mass transfer is much weaker than in case B: the supernova progenitor must still be originally the less massive of the two, but its mass may differ from that of the original primary by as much as  $\sim 10\%$ . In this case, mass transfer occurs when the original primary reaches the asymptotic giant branch (after the completion of helium core-burning), whereas the supernova progenitor can be in any preceding post-main-sequence phase. The original primary then explodes as a supernova shortly after the mass-transfer phase and should leave a neutron-star remnant (this explosion occurred in the rather distant past and should not be confused with the observed supernova event). The binary system remains bound but acquires a small eccentricity,  $e \approx 0.1-0.2$ . For up to some  $10^6$  yr after-

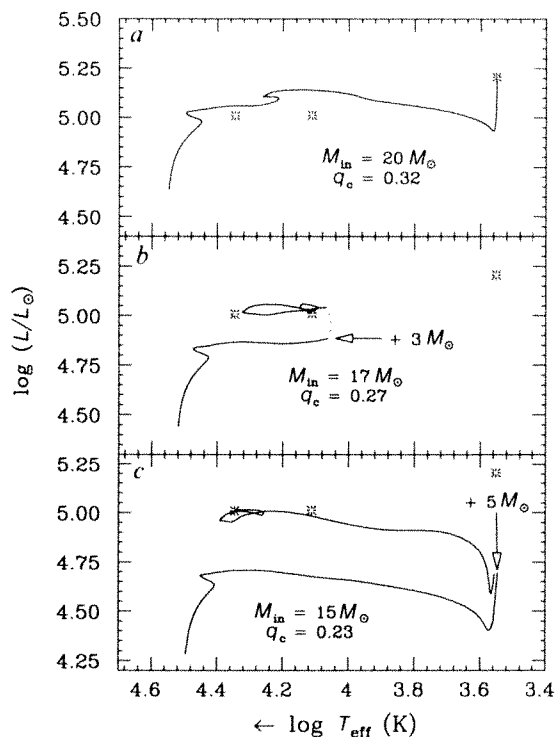


FIG. 1 Evolutionary tracks of the progenitor in the Hertzsprung-Russell diagram (stellar luminosity,  $L$ , versus effective temperature,  $T_{\text{eff}}$ ) for several assumed amounts of accreted mass. *a*, Evolutionary track of a 20- $M_{\odot}$  star without accretion. *b*, Evolutionary track of a star with an initial mass of 17  $M_{\odot}$  which accretes 3  $M_{\odot}$  after the termination of core hydrogen burning (the dotted portion of the curve denotes the mass-transfer phase; the arrow indicates the onset of mass transfer). *c*, Evolutionary track for a star with an initial mass of 15  $M_{\odot}$  which accretes 5  $M_{\odot}$  after the core hydrogen-burning phase; here, the small break in the curve denotes the mass-transfer phase. In each case,  $M_{\text{in}}$  denotes the initial mass and  $q_c \equiv M_c/M$  is the final fractional core mass,  $M_c$  being the mass of the hydrogen-exhausted core and  $M = 20 M_{\odot}$ , the final mass of the progenitor. The asterisks indicate the parameters of the models just before the supernova event. For comparison, the final parameters of all three cases are shown in each panel.

wards, while the supernova progenitor undergoes advanced evolutionary phases, the neutron star will accrete a portion of any stellar wind emitted by the progenitor. Using the Bondi-Hoyle model<sup>20</sup> for wind accretion, we find that the expected X-ray luminosity,  $L_X \approx 1 \times 10^{34}$  ergs s<sup>-1</sup>, is well below the upper limit for the X-ray luminosity in the direction of SN1987A obtained from archival Einstein data<sup>21</sup> ( $L_X \leq 9 \times 10^{34}$  ergs s<sup>-1</sup>).

More than half the total mass of the binary system will have been ejected in the observed supernova explosion, and the system will now be unbound. The older neutron star (the collapsed remnant of the original primary) may become detectable within the next few years, either as a radio pulsar or as an X-ray source powered by the accretion of material from the supernova remnant.

The principal advantage of either of the above scenarios over single-star models for SN1987A is that a blue progenitor is a natural consequence of conventional binary stellar evolution theory, and does not depend on *ad hoc* assumptions or modifications of the input physics. Moreover, our binary model may account for many of the anomalous features of SN1987A (see also ref. 2). In particular, the natural structure of our pre-supernova model is sufficiently similar to those of single-star models<sup>15,22,23</sup> that we can expect fits to the supernova light curve entirely as good as those achieved in the single-star models. The asymmetric expansion of the ejecta, as inferred from polarization measurements and speckle interferometry<sup>24,25</sup>, also has a natural explanation in our model, as we expect the progenitor to be rotationally flattened as a result of the spin-up it experienced during the mass accretion phase (see also R. A. Chevalier, preprint). Moreover, the relativistic-jet models<sup>26</sup> that have been invoked to explain the 'mystery spot'<sup>27</sup> seem to require a close-binary companion, and the existence of a nearby companion star may account for the observed variability<sup>28</sup> of the soft X-ray emission (ref. 29 and R. McCray, personal communication).

Our model also provides a plausible mechanism to explain the anomalously high abundance of barium and other s-process elements in the ejecta<sup>30</sup>. If the companion passed through a helium-giant phase<sup>31</sup>, s-processed material might have been dredged up<sup>32</sup> and subsequently transferred to the progenitor by Roche-lobe overflow. Alternatively, mass transfer might spin up the progenitor nearly to break-up velocity, in which case meridional currents might overcome the stabilizing effect of chemical gradients<sup>33</sup> and produce large-scale mixing of the envelope, including the dredge-up of s-processed material (see also refs 34 and 35). We note that there is no known mechanism to account for this anomaly in single-star scenarios for SN1987A and that all known barium stars are found in binary systems<sup>36</sup>.

Future observations of the supernova and its environment (such as the nitrogen-rich circumstellar material<sup>37</sup>) will help to distinguish between the single star and the binary models. However, the ultimate test will be the reappearance of the predicted companion star, be it a relatively normal star, a neutron star, or Sk-69°202 itself. We should know within a few years whether SN1987A was a member of a binary system and, if so, which of the proposed binary models is correct. □

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## Stabilization of lamellar oil-water liquid crystals by surfactant/co-surfactant monolayers

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LIQUID crystals are divided into two main classes, thermotropic and lyotropic. Thermotropic liquid crystals are formed by melting, whereas lyotropic liquid crystals arise from the association of molecules, such as soap and water, that in general are not in themselves liquid crystalline. Thermotropic liquid crystals are used for liquid-crystal displays; lyotropic liquid crystals occur in living cells. Here we report a novel sequence of lyotropic liquid crystals comprising alternate layers of oil and water whose thickness varies linearly with the relative proportions of oil and water, and we have determined their structure using neutron diffraction methods. The oil and water layers are separated and stabilized by a monolayer film of surfactant and co-surfactant. The individual layers are typically a hundred ångströms or more in thickness, and total lamellar spacings of up to 1,000 Å were observed. This behaviour is difficult to describe in terms of the theories of colloid stability currently used to describe lyotropic liquid crystals. An understanding of the self-organization of such systems over such large distances would elucidate how long-range liquid-crystalline ordering arises in living cells. Moreover, thermotropic liquid crystals are expensive and chemically relatively unstable, and lamellar mesophases of the lyotropic type described here could lead to inexpensive, chemically stable liquid-crystalline materials suitable for industrial application.

Lamellar mesophases of surfactant/water liquid crystals<sup>1</sup> are well characterized structures comprising alternate surfactant bilayer sheets and water layers about 30 Å thick. Diluting these mesophases rarely results in lamellar repeat spacing of more than 100 Å; instead, either a micellar phase is formed or phase separation occurs. Dispersions of oil and water stabilized by surfactant and co-surfactant frequently form fluid isotropic microemulsions<sup>2</sup>. Under some circumstances, however, such

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mixtures can give rise to liquid-crystalline phases that have lamellar, hexagonal or cubic structures<sup>3-5</sup>. We have studied the lamellar mesophases prepared from tetradecyltrimethylammonium bromide (TTAB,  $C_{14}H_{29}N^+(CH_3)_3Br^-$ ) as surfactant, pentanol as co-surfactant, cyclohexane and water. Three series of compositions were studied: in series A and B, the amounts of surfactant and co-surfactant were constant, as was the total volume of cyclohexane and water, but the relative volume fraction of cyclohexane to water was varied. In series C, the lamellar phase was gradually diluted with an aqueous solution of 1% by weight of sodium bromide pre-saturated with pentanol and cyclohexane. The compositions were as follows: (A) 0.70 g TTAB, 0.38 ml pentanol, 4.0 ml of water and cyclohexane; (B) 0.29 g TTAB, 0.33 ml pentanol, 4.2 ml of water and cyclohexane; (C) TTAB/pentanol/cyclohexane in the weight ratio 1:0.56:0.55 in a 1% by weight solution of NaBr pre-saturated with cyclohexane and pentanol. For composition C the overall TTAB concentration varied from that used in composition B down to one eighth of this value.

In the absence of cyclohexane, composition A formed a birefringent phase which persisted on replacement of water by oil for oil-to-water volume fractions of 90% cyclohexane/10% water. Composition B was not liquid crystalline in the absence of oil but formed a birefringent phase as soon as a small amount of cyclohexane was added. The birefringent phase was stable for samples containing up to 70% cyclohexane/30% water, after which phase separation occurred into optically birefringent and isotropic phases. For surfactant concentrations much less than those used in series B, the oil-in-water lamellar phases became unstable. Stable lamellar phases down to much lower concentrations were obtained by using an aqueous solution of NaBr instead of water.

All the birefringent phases gave neutron diffraction patterns consistent with a lamellar structure, and up to three diffraction orders were observed. For compositions A and B, the overall repeat spacings were 130 Å and 230 Å respectively. The lamellar mesophases orientate in magnetic fields; Fig. 1 shows the neutron intensity distribution over a two-dimensional detector from a typical orientated sample. Neutron-scattering-length densities of protonated and deuterated materials are of very different magnitude. Hence, in multicomponent systems, selective deuteration of different components can be used to highlight

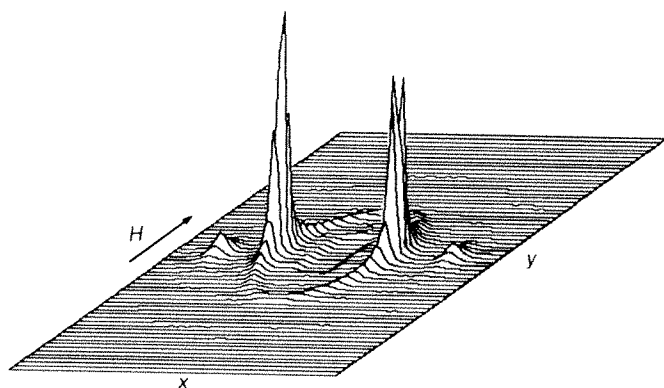


FIG. 1 Neutron scattering pattern on a two-dimensional  $64 \times 64$  cm detector for an orientated mesophase with an oil-to-water volume fraction of 45% cyclohexane/55%  $D_2O$ . The sample composition was 0.70 g tetradecyltrimethylammonium bromide, 0.38 ml pentanol, 1.80 ml cyclohexane and 2.20 ml  $D_2O$ . All measurements were done on the D17 diffractometer<sup>17</sup> at the Institut Laue-Langevin, Grenoble, France. The pattern was recorded in 20 min using a sample-to-detector distance of 1.40 m and a wavelength of 9.0 Å. The neutron beam, stopped by a rectangular cadmium plate after transmission through the sample, is centred on the detector; two reflections on either side correspond to the first and second lamellar diffraction orders. Orientation was achieved by cooling the sample from 60 °C to 20 °C in a magnetic field of 6.5 tesla over 4 h. Shown in the figure is the direction of the magnetic field,  $H$ , with respect to the detector.

specific features of the structure. Figure 2 shows a Fourier profile, derived from the phased structure factors using standard procedures<sup>6</sup>, for a mesophase of composition A. The profile corresponds to a low-resolution image of the variation in neutron-scattering-length density normal to the lamellae. Both the water and cyclohexane were deuterated; the maxima correspond to the layers of deuterated oil and water, and minima correspond to the protonated surfactant/co-surfactant layers. Fourier profiles obtained for samples containing either surfactant or pentanol as the only protonated components were nearly identical to those in Fig. 2, indicating that these two components share a common location within the structure. Most of the pentanol molecules are therefore intercalated between the surfactant chains. Deuteration of oil or water reduces alternate maxima, corresponding to either the protonated oil or water layers, to the level of the protonated surfactant/co-surfactant minima. For samples consisting of  $D_2O$ , deuterated cyclohexane, deuterated pentanol and  $C_{14}D_{29}N(CH_3)_3Br^-$ , the scattering arises solely from the surfactant polar heads. The thickness of the surfactant/co-surfactant layer was determined as  $\sim 17$  Å, equal to half of the bilayer thickness derived from samples containing no cyclohexane. These results show that the lamellar structure comprises alternating layers of cyclohexane and water separated and stabilized by a monolayer film of surfactant and co-surfactant. As the cyclohexane-to-water volume fraction increases, there is no change in the total repeat spacing but the cyclohexane layer thickens at the expense of the water region. Shown in Fig. 3 are plots of the overall spacing and the thicknesses of cyclohexane and water layers versus the cyclohexane-to-water volume fraction. The cyclohexane layer thickness increases almost linearly with oil content, and a corresponding decrease occurs in the  $D_2O$  layers, until for composition A at 90% cyclohexane/10%  $D_2O$  the  $D_2O$  layer thickness is  $\sim 10$  Å. Similar results were obtained for composition B, for which the overall repeat-unit thickness was 230 Å, and the cyclohexane and  $D_2O$  layer thicknesses, just before phase separation (at an oil-to-water volume fraction of greater than 70% cyclohexane/30%  $D_2O$ ), were  $\sim 110$  Å and  $\sim 80$  Å, respectively. For

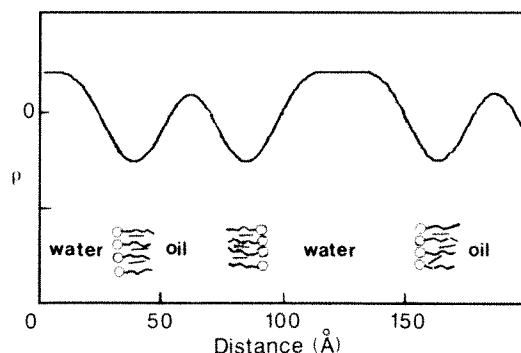


FIG. 2 Scattering-length density profile at  $20.0 \pm 0.2$  °C for a mesophase composed of 0.70 g tetradecyltrimethylammonium bromide, 0.38 ml pentanol, 1.4 ml deuterated cyclohexane and 2.6 ml  $D_2O$ . The repeat spacing is  $125 \pm 2$  Å. Also shown is a schematic drawing of the structure, with pentanol molecules represented as dark rods intercalated between surfactant molecules. The Fourier profiles were calculated from  $\rho(x) = \pm |F_n| \cos(2\pi nx/d)$ , where  $\rho(x)$  is the coherent scattering amplitude density,  $|F_n|$  is the structure-factor modulus of the  $n$ th order diffraction peak and  $d$  is the repeat spacing. Because preparations with different oil-to-water volume ratios gave different degrees of magnetic orientation, non-orientated samples were used, and structure factors were calculated from the diffraction intensities  $I_n$  according to  $|F_n| = (\sin 2\theta_n \sin \theta_n)^{1/2} I_n^{1/2}$ , where  $(\sin 2\theta_n \sin \theta_n)$  is the Lorentz factor as applied to randomly orientated or powder samples. Phases—equal to either zero or  $\pi$  for lamellar structures—were determined by substituting deuterated for protonated molecular components and by swelling the structure (by changing the temperature). Diffraction patterns were recorded typically in 60 min on the diffractometer D16 at the Institut Laue-Langevin<sup>11</sup>. Statistical errors in the structure factors were better than  $\pm 1\%$  for the strong reflections and a few per cent for the weak peaks.

composition C (1% NaBr solution), samples containing much less surfactant and co-surfactant were strongly birefringent, and the observed lamellar spacings ranged up to 1,000 Å.

In most surfactant-water mixtures, lamellar phases do not arise until concentrations of ~50% by weight are attained, such that the thicknesses of the surfactant bilayer and the water layer are comparable (~30 Å). The addition of a co-surfactant such as pentanol promotes the formation of lamellar phases at lower surfactant concentrations, giving higher spacings. For example, in the absence of cyclohexane, composition A forms a lamellar phase which persists down to surfactant concentrations of ~10% by weight. At lower concentrations, micelles are formed. This behaviour can be explained in the following manner. The pentanol molecules are intercalated between the surfactant hydrocarbon chains, reducing the curvature of the micelle-water interface until flat surfaces are formed and a lamellar packing results. The addition of both co-surfactant and oil favours the formation of a lamellar phase for surfactant concentrations even lower than 10% by weight; by adding KBr, lamellar phases formed at less than 1% of surfactant. Once a flat monolayer has been formed, the lamellar structure may be retained whilst significantly modifying the relative proportions of oil and water. There has been considerable debate concerning the structure of microemulsions containing equal volumes of oil and water<sup>7-9</sup>. One possibility comprises bicontinuous structures of cubic symmetry in which the local surface curvature is non-zero but the total curvature is very low<sup>3,7-9</sup>. Another possibility is the lamellar structures such as those reported here. It should be possible to effect a transition between the two types of structure by varying the co-surfactant content and thus modifying the local interfacial curvature.

These arguments based on interfacial curvature may help to explain why these lamellar structures arise. However, the nature of the forces operative in this system, for which aqueous-based or oil-rich mesophases can be prepared with neither loss of the lamellar structure nor a change in the repeat structure, remains to be determined. The stability of lamellar mesophases relative to other structures may be understood classically in terms of repulsive electrostatic interactions and attractive van der Waals forces<sup>10,11</sup>. Both of these contributions are strongly modified by replacing water by oil and both decay rapidly with increasing distance. Electrostatic interactions decay exponentially with the inverse Debye length. The Debye lengths calculated for the systems with no added NaBr varied from ~5 to 10 Å, and were less than 10 Å for the systems with added NaBr. Classical theories of electrostatics do not seem to account for the observed

behaviour; in particular, decreasing the Debye length by adding salt results in a stabilization of the lamellar phase to longer layer spacings. This, and the existence of a lamellar structure over a wide range of oil-to-water volume ratios, suggests that additional long-range forces might be operating. An interfacial-fluctuation-driven mechanism<sup>12</sup> has been suggested in this respect, but the applicability of this mechanism has not been established, and it is difficult to see how it can explain recent results obtained on clay/water systems<sup>13</sup>. For aqueous layers between hydrophobic surfaces, Israelachvili and Pashley *et al.*<sup>14,15</sup> deduced the presence of strong long-range attractive hydrophobic forces, which are difficult to explain in classical terms. If such long-range forces exist, then they would be of considerable importance in many areas of molecular biology and colloid science. The lamellar mesophases presented here may be suitable systems in which to probe their nature, by, for example, force-distance measurements. These lamellar oil-water mesophases may find several applications<sup>16</sup>. In particular, lyotropic liquid crystals with a range of tailored properties may be prepared by the inclusion of appropriate compounds or colloidal materials into the aqueous, hydrocarbon or interfacial regions. Structural studies of the type reported here are an essential step towards the development and exploitation of this novel technology. □

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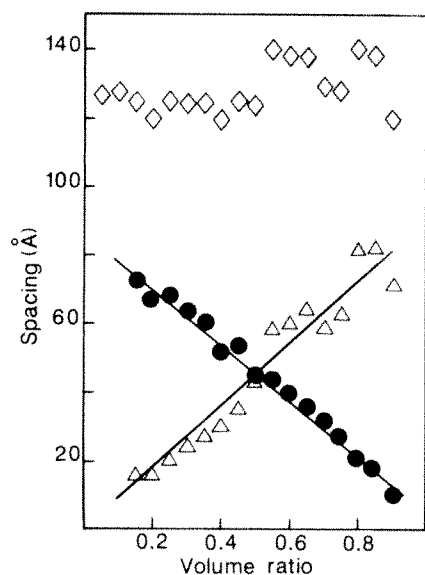


FIG. 3 Plots of repeat spacing (◇), and water (●) and cyclohexane (△) layer thicknesses, as a function of oil-to-water volume ratio, for composition A.

## Atmospheric <sup>14</sup>C and century-scale solar oscillations

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THE solar-wind plasma in our interplanetary space deflects part of the Earth-bound cosmic-ray flux through its magnetic interaction with the electrically charged incoming particles. Because changes in the magnetic properties of the plasma originate at the Sun's surface, the cosmic-ray flux arriving at Earth therefore depends on the changing surface conditions of the Sun. Consequently, by monitoring the variable production rate of cosmogenic isotopes (such as <sup>14</sup>C) in our atmosphere, a time history of changing conditions of the Sun's surface can be obtained. Trees, through carbon dioxide assimilation, lay down a <sup>14</sup>C record which provides clues towards the causes underlying <sup>14</sup>C production-rate changes. Here we show from maximum-entropy spectral analysis of a 9,600-yr high-precision <sup>14</sup>C chronology that changes occur in the Sun's convective zone with a fundamental oscillatory mode of about  $2.4 \times 10^{-3} \text{ yr}^{-1}$  (420-yr period), and we also identify several harmonics. Previous searches<sup>1-3</sup> for cyclicity in the atmospheric <sup>14</sup>C record have yielded periods near 140 and 200 yr. We discuss the implications of a longer and more precise <sup>14</sup>C record.

Determinations for this study were made by the precise counting of the  $^{14}\text{C}$  radioactivity of large samples, the sample activity being compared with the absolute National Bureau of Standards (NBS) oxalic acid standard activity. For tree-ring materials of known age a correction for  $^{14}\text{C}$  decay is applied. The  $^{14}\text{C}$  results are also normalized on a fixed  $\delta^{13}\text{C}$  value of  $-25\%$  relative to the PDB standard

$$\left(\delta^{13}\text{C} = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{PDB}}} - 1 \right] 1,000\% \right)$$

The results are expressed as  $\Delta^{14}\text{C}$  (ref. 4), which represents the  $^{13}\text{C}$ -normalized  $^{14}\text{C}$  activity at the time of formation, expressed as the deviation in parts per thousand from the oxalic acid standard activity.

A compilation of the tree-ring  $^{14}\text{C}$  data reported in ref. 5 yielded the basic 9,600-yr bi-decadal  $\Delta^{14}\text{C}$  record used here. The data of the Seattle<sup>6,7</sup> and Belfast<sup>7,8</sup> laboratories extend back to 5,200 yr BC, whereas a mixture of results from Seattle<sup>9</sup>, La Jolla<sup>10</sup>, Tucson<sup>11</sup> and Heidelberg<sup>12</sup> was used for the interval 5200–7750 BC. The Seattle–Belfast portions of the data set have a typical standard deviation of 1.5–2% for bi-decadal wood. Such precision includes overall reproducibility as well as the Poisson error in the number of counts. For pre-5200 BC data, the average standard deviation derived from the scatter of the results reported within 20-yr blocks is 5%.

The long-term  $\Delta^{14}\text{C}$  record shows a broad 10% decline towards the present, which is usually attributed to the influence either of an increasing geomagnetic dipole moment on  $^{14}\text{C}$  production rate or of changing carbon-reservoir parameters (that is, climatic conditions) on atmospheric  $^{14}\text{C}$ . Because we focus attention here on solar modulation of the cosmic-ray flux, the long-term trend was removed by subtracting a spline approximating a 400-yr moving average. Our contention that most, if not all, of the century-scale oscillations in the residual  $\Delta^{14}\text{C}$  record (Fig. 1) are heliomagnetic in origin is supported by calculations of the magnitude of the expected solar-induced  $\Delta^{14}\text{C}$  variations<sup>13</sup>, by the coincidence of the  $\Delta^{14}\text{C}$  maximum near AD 1700 with the lack of sunspots experienced during the AD 1654–1714 Maunder minimum<sup>13,14</sup>, by the compatibility of the  $^{14}\text{C}$  history of the last millennium with auroral evidence and naked-eye sunspot observations<sup>13,15</sup> and by the similarity (in timing as well as magnitude) of the  $^{10}\text{Be}$  cosmogenic record in ice cores and the atmospheric  $\Delta^{14}\text{C}$  record<sup>16</sup>.

The  $\Delta^{14}\text{C}$  oscillations of a few per cent in Fig. 1 have approximately equal rates of  $\Delta^{14}\text{C}$  increase and decrease. They differ slightly in length: the Maunder oscillations have a period of  $\sim 180$  yr and the Spörer oscillations last  $\sim 40$  yr longer<sup>17</sup>. These oscillations are denoted M and S in Fig. 1. Nine of the Maunder-type oscillations and eight of the Spörer variety are found in the 9,600-yr record. Episodes of triple oscillations, discussed later, are labelled  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$ .

The atmospheric  $^{14}\text{C}$  signal results from frequency-dependent attenuation of variations in  $^{14}\text{C}$  production rate,  $Q$ , and so is a less direct record of heliomagnetic change than is  $Q$  itself. We calculated  $Q$  from the observed  $\Delta^{14}\text{C}$  record by carbon-reservoir modelling<sup>13,17</sup>, and determined the spectral distribution of the entire 9,600-yr  $Q$  record, without removing any trends. From a Fourier analysis, spectral power exceeding the  $2\sigma$  significance level<sup>18</sup> was found at periods of 230–200, 150, 88 and 57 yr. The maximum entropy method (MEM or autoregressive (AR) model), using the Burg<sup>19</sup> or FABNE<sup>20</sup> algorithms and AR order 20, yields spectral power for similar periods (Fig. 2). The minimum final-prediction error (FPE) was the selection criterion for AR order 20 (ref. 21). Virtually the same set of cycles (425, 227, 148, 85, 67, 58 and 45 yr) is found through the MEM approach (AR order 20) after de-trending  $Q$  in the same manner as shown in Fig. 1 for the atmospheric  $\Delta^{14}\text{C}$  record.

A set of harmonics underlies the periods in Fig. 2, as demonstrated by the inset, in which the hyperbola represents the

harmonics of the 420-yr period. The fourth harmonic, with a calculated period of 105 yr, is not present in the entire spectrum of Fig. 2, but on subdividing the record into four equal parts we find 110- and 107-yr cycles in, respectively, the 5350 BC–2970 BC and 570 BC–AD 1830 portions.

The cycles with 67-, 52- and 45-yr periods contain about one twentieth of the spectral power of the longer cycles in the Fourier spectrum. They may be related to the non-sinusoidal shape of the slower cycles. For instance, a square wave with a 420-yr period generates harmonics in our fast-Fourier-transform spectrum at about one twentieth of the strength of the 420-yr peak at periods of 139, 84, 60 and 47 yr.

The harmonics shown in Fig. 2 express the average oscillatory properties of the 9,600-yr record. A least-squares procedure was used to determine phase relationships and relative amplitudes for each of the principal oscillations (420, 218, 143 yr) generated by the MEM method. The iterative procedure involved testing combinations of phases and amplitudes for periods near each of the principal oscillation periods until a best fit with the observed record was achieved. The relative phases of the solar

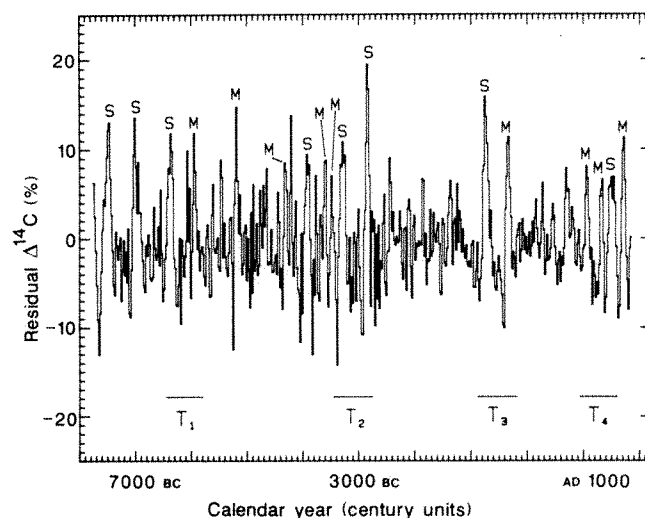


FIG. 1 Residual atmospheric  $\Delta^{14}\text{C}$ , obtained by deducting the long-term  $\Delta^{14}\text{C}$  trend. M and S denote Maunder- and Spörer-type events. Episodes of triple oscillations ( $T_1$ – $T_4$ ) are discussed in the text.

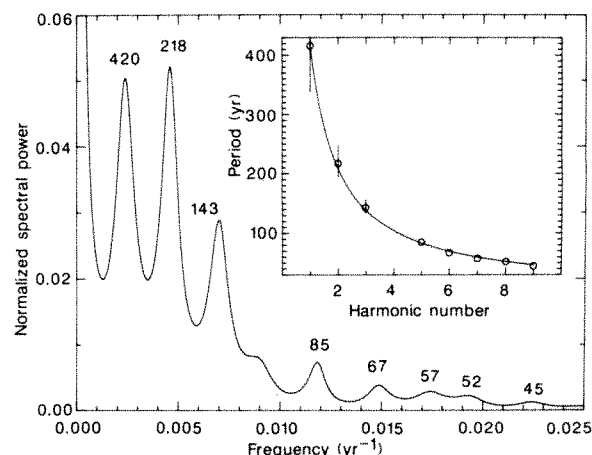


FIG. 2 MEM power spectrum (AR=20) of  $^{14}\text{C}$  production rate  $Q$ . The periods are listed with the individual peaks. The hyperbola in the inset shows the harmonics expected for a fundamental frequency of  $1/420 \text{ yr}^{-1}$ . The observed periods are plotted in the inset with vertical bars representing one standard deviation (error bars smaller than the symbol size are not shown).



periodicities were assumed to remain unchanged for the entire 9,600-yr record. The periods obtained in this manner differ slightly from those in the raw MEM analysis.

Improved coherence is obtained for different sections of the record when two variants of the average (420, 218, 143 yr) mode are considered. Sections of the record ( $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$  in Fig. 1), each of which contains at least two of the Maunder- and Spörer-type digressions, show clear similarities when viewed on an amplified timescale<sup>17</sup>. A best fit to these segments, retaining their relative timing, yields equation (1), whereas the remaining sections are best represented by equation (2):

$$\frac{\Delta Q}{Q_t}(\%) = 8.8 \cos\left(\frac{2\pi}{435}t + 1.5\pi\right) + 11.2 \cos\left(\frac{2\pi}{207}t + 1.6\pi\right) + 5.6 \cos\left(\frac{2\pi}{148}t + 0.5\pi\right) \quad (1)$$

$$\frac{\Delta Q}{Q_t}(\%) = 2.8 \cos\left(\frac{2\pi}{456}t + \pi\right) + 4.3 \cos\left(\frac{2\pi}{256}t + 0.8\pi\right) + 3.4 \cos\left(\frac{2\pi}{143}t + 0.3\pi\right) \quad (2)$$

with  $\Delta Q/Q_t$  being the percentage deviation of  $Q$  from the splined long-term trend  $Q_t$ , and  $t$  being time in calendar years (negative for dates BC).

In Fig. 3b the solid curve shows the correlation coefficients between sliding 700-yr portions of equation (1) (435, 207, 148 variant) and the  $^{14}\text{C}$  production record, and the dotted curve gives the corresponding correlation for equation (2) (456, 256, 143 variant). The first variant generates the basic pattern of the four triplets  $T_1$ – $T_4$  without changing phase or amplitude of the sine waves (Fig. 3a).

The discussion so far has centred on the periodicities generated by the MEM method using moderate AR numbers. By increasing the AR order or using Fourier power spectra, additional periods become important. For instance, the single 420-yr peak (Fig. 2) splits into 504-, 355- and 299-yr peaks in the Fourier analysis of the complete de-trended  $Q$  record. Spectral properties also vary to some extent for different parts of the record (for example, in the 570 BC–AD 1830 quarter section a pronounced 127-yr peak, absent in the other sections, is observed).

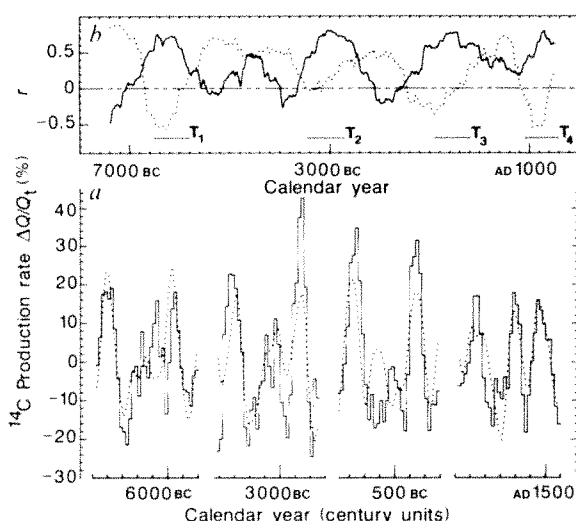


FIG. 3 a, Tree-ring-derived changes in  $^{14}\text{C}$  production rate  $Q$  of the triplet episodes  $T_1$ – $T_4$  (solid line), compared with the curve generated from equation (1) (dotted line). Triplet intervals are 6480–5800 BC, 3420–2740 BC, 880–200 BC and AD 920–1600. b, Correlation coefficient  $r$  of a 700-yr sliding portion of the  $^{14}\text{C}$  production rate with curves generated from either equation (1) (solid line) or equation (2) (dotted line). The 700-yr sliding window results in a loss of 350 yr at either end of the curves.

Adding frequencies to equation (1) and using variable amplitude or phase shift would improve the correlation, but our goal here was to explain the major features of the  $^{14}\text{C}$  record in the least complicated manner.

Although we have argued that the main century-scale atmospheric  $^{14}\text{C}$  oscillations are best explained in terms of variations in  $^{14}\text{C}$  production rate induced by solar change, we have also considered the oceanic changes that would be required to produce these atmospheric patterns. Atmospheric  $\Delta^{14}\text{C}$  can increase either by a reduction in the  $\text{CO}_2$  gas exchange rate at the air-sea interface or by a reduction in the rate of upwelling of  $^{14}\text{C}$ -deficient deep ocean water. Carbon-reservoir modelling indicates that global wind speeds would have to be reduced to two-thirds of the current average to create a Maunder  $\Delta^{14}\text{C}$  event; alternatively, mixing rates in the global ocean would have to fall back rapidly to half the long-term average. Such changes are large, and there are no proxy data to support such extreme behaviour. Thus we consider that the  $\Delta^{14}\text{C}$  oscillations are best explained by solar-wind forcing.

Evidence of global solar change is provided by the 22-yr Hale (sunspot) cycle, from which a 90-yr (Gleissberg) periodicity has been deduced. Analysis of the number of aurorae reported per decade gives periodicities of 88 and 130 yr (refs 23, 24). The 88-yr periodicity is near the 85–87-yr period derived from the  $^{14}\text{C}$  data (Fig. 2), whereas the 130-yr period is close to the 127-yr period found for the 570 BC–AD 1830 interval. Our present analysis adds periodicities of about 140, 220 and 420 yr to these observations of solar patterns.

Solar-constant changes of up to 0.1% have been measured for the recent part of the Hale cycle<sup>25,26</sup>. Evidently the changes that result in sunspot and cosmic-ray modulation also induce changes in the solar constant. The longer periodicities of the  $^{14}\text{C}$  production record may also relate to such solar-constant change, and these changes may be larger in magnitude. If so, a relationship between climate and solar variability, as evidenced in the  $^{14}\text{C}$  record, is to be expected. Conflicting evidence exists for such a relationship<sup>27</sup>. Strong evidence for a correlation was given by Sonett and Suess<sup>22</sup> for a tree (bristlecone pine) ring-width record, for which periodicities of, for example, 114 and 208 yr were found.

Applying AR techniques to ten climate records (tree-rings and glacier fluctuations) reported by Röthlisberger<sup>28</sup>, we find that none of the spectra contain significant power in the range 200–250 yr. However, there is power in the 123–143-yr interval for six of the records, in the 102–104-yr range for three, and near 88 yr for two. In our previous study<sup>1</sup> of climate records covering only one millennium we found no convincing evidence for a Sun-climate relationship. The use of the much longer Röthlisberger records together with the  $^{14}\text{C}$  analysis reopens this question, and in particular there may be a Sun-climate relationship for the third harmonic (140 yr). □

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## Influence of aqueous aluminium and organic acids on measurement of acid neutralizing capacity in surface waters

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ACID neutralizing capacity (ANC) is used to quantify the acid-base status of surface waters. Acidic waters have been defined as having ANC values less than zero, and acidification is often quantified by decreases in ANC. Measured and calculated values of ANC generally agree, except for low-ANC waters. These waters, however, are of primary interest in lake-acidification studies. The discrepancy in ANC values is greatest for waters with high concentrations of aluminium and/or dissolved organic carbon (DOC). The discrepancy due to aluminium increases with increasing concentration of dissolved monomeric aluminium ( $Al_m$ ) and can exceed  $50 \mu\text{eq l}^{-1}$  at low pH and high  $Al_m$  values. Here we show that this error can be minimized by using a proton reference level for aqueous aluminium of 2+, rather than 0 or 3+, as is done usually. The value of 2+ is consistent with the mean charge on aluminium at the equivalence point of strong-acid titrations. The discrepancy between calculated and measured ANC also increases with increasing DOC concentrations, exceeding  $50 \mu\text{eq l}^{-1}$  for waters with DOC concentrations greater than  $800 \mu\text{mol C l}^{-1}$  ( $1000 \mu\text{mol C l}^{-1} = 12 \text{ mg l}^{-1} \text{ DOC}$ ). This error can be decreased by titrating to a low consistent pH value (for example, 3.0). This reduces the systematic underestimation of ANC due to curvature in Gran<sup>1</sup> plot analysis, while still allowing accurate measurement of increments in hydrogen-ion concentration. ANC should not be used as a single parameter for characterizing the chemical suitability of surface waters for biota or for assessing the susceptibility of low-ANC waters to acidification by acid deposition.

In regional investigations of acid-base status, ANC has been the principal classification variable<sup>2</sup>. ANC is also widely used by simulation models that predict the response of ecosystems to changes in atmospheric acid deposition<sup>3–7</sup>. Historical changes in surface-water quality have been evaluated using measured changes in ANC<sup>8</sup> or estimated by inferring past and present pH and ANC from lake sediment diatom assemblages<sup>9,10</sup>.

ANC has also been used extensively to assess the susceptibility of surface waters to atmospherically derived strong acids<sup>11,12</sup>. Waters with high ANC values would generally be considered resistant to changes in pH resulting from changes in deposition acidity. Low-ANC waters may or may not resist changes in pH, depending on the processes supplying ANC in the terrestrial or aquatic environment<sup>13</sup>.

ANC has been used as a primary indicator of a water's suitability for aquatic biota. Other water-quality parameters, however, also need to be considered, including pH (ref. 14), inorganic monomeric aluminium<sup>14</sup>, and calcium<sup>15</sup>, as well as habitat characteristics (such as the availability of spawning substrate). In low-ANC waters, inorganic monomeric aluminium may constitute a large fraction of the non-hydrogen cations (see below) and thus be of biological significance.

ANC is a measure of the net strong base in solution. It is measured by quantifying the amount of strong acid that must be added to a solution to neutralize this base. The end point of this strong-acid titration would be easily identified except for the presence of weak acids and the relatively small amounts of strong base present in low-ANC waters. Together, these factors obscure the end point. For such systems, the Gran<sup>1</sup> procedure is most commonly used to determine the end point and therefore ANC.

ANC can be calculated by two distinct methods, which have been shown to be mathematically equivalent using the principles of conservation of charge and conservation of mass<sup>16</sup>. In one method<sup>17</sup>, ANC is calculated as the difference between the sum of the proton ( $H^+$ -ion) acceptors and the sum of the proton donors, relative to a selected proton reference level (discussed below):

$$\text{ANC} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] + [\text{other proton acceptors}] - [\text{H}^+] \quad (1)$$

Here, square brackets denote molar concentrations. The other method relates ANC to the total non-hydrogen cation concentrations ( $M_T$ ; free and complexed forms); the individual uncomplexed cation charges ( $z_i$ ) at the equivalence point (the point at which, during titration, the concentration of proton donors equals the concentration of proton acceptors); the total strong-acid anion concentrations ( $A_T$ ; free and complexed); and the individual uncomplexed anion charges ( $z_j$ ), at the equivalence point (general theory<sup>16</sup>; practice<sup>18,19</sup>) using the following relation:

$$\text{ANC} = \sum_i^n |z_i| M_{T_i} - \sum_j^m |z_j| A_{T_j} \quad (2)$$

For aerobic surface waters the first term on the right hand side of equation (2) is typically approximated as

$$\sum_i^n |z_i| M_{T_i} \approx 2[\text{Ca}_T] + 2[\text{Mg}_T] + 2[\text{Mn}_T] + [\text{K}_T] + [\text{Na}_T] + [\text{NH}_4] + x[\text{Al}_T] \quad (3)$$

and the second term is approximated as

$$\sum_j^m |z_j| A_{T_j} \approx 2[\text{SO}_{4T}] + [\text{NO}_{3T}] + [\text{Cl}_T] + [\text{F}_T] \quad (4)$$

The charges  $z_i$  and  $z_j$  (and thus the concentration multipliers in equations (3) and (4)) are determined by the predominant charges of the uncomplexed constituents at the equivalence point.

For most of the species, there is little uncertainty as to the predominant uncomplexed charge at the equivalence point. For example, the charge of calcium is 2+, and thus the multiplier is 2 in equation (3). However, because of complexation with OH, F and organic ligands, the charge of aluminium, shown as  $x$  in equation (3), is not always obvious. Designation of this charge, however, establishes the proton reference level (PRL).

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PRL constituents do not change ANC but may change pH when added to waters isolated from soils and sediments<sup>20</sup>. In the literature<sup>5,18,19</sup>, two PRLs have been used for aluminium, 3+ and 0. These levels have different advantages; the former yields results that are closer to Gran ANC values; the latter eliminates the need to include aluminium in ANC calculations.

Data collected during the regionalized integrated lake-watershed acidification study (RILWAS)<sup>21,22</sup> from 25 lake-watershed systems in the Adirondack mountains of New York were used

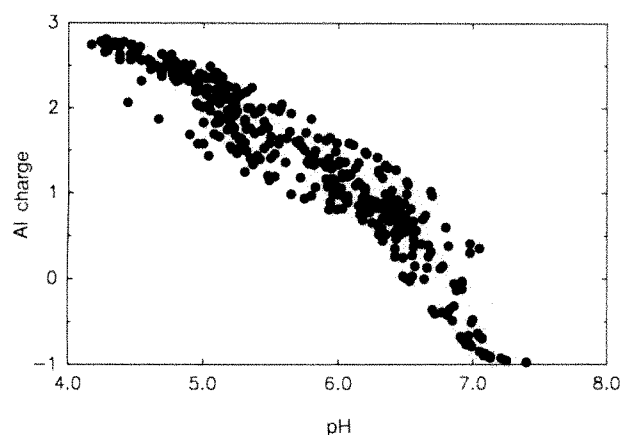


FIG. 1 Mean charge of monomeric inorganic Al versus pH for RILWAS waters. Equivalence point of Gran titrations for dilute waters generally occurs in pH range 4.8 to 5.2. Here the equivalent charge of the aluminium is near 2+.

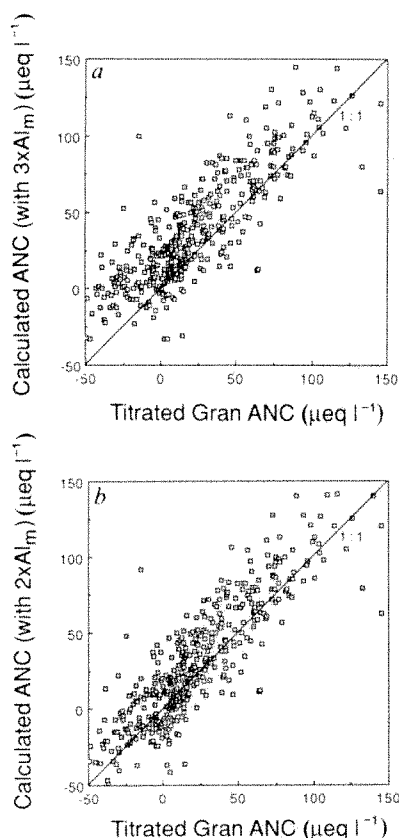


FIG. 2 Calculated ANC versus titrated Gran ANC for RILWAS lakes: Calculated ANC using a, proton reference level for  $Al_m$  of 3+ and b, proton reference level of 2+.

to estimate the aluminium PRL. The speciation of aluminium was calculated using the chemical equilibrium model ALCHEMI<sup>23</sup>, and the equivalent charge on the aluminium species was determined (Fig. 1). The mean charge on the aluminium increases with decreasing pH. However, over the pH range from 4.8 to 5.2, which corresponds to the equivalence point of dilute waters<sup>24</sup>, an aluminium charge of 2+ appears more representative than 3+ or zero. Moreover, Gran ANC more closely follows calculated ANC where two times  $Al_m$  is used in the calculation for RILWAS lakes (Fig. 2). This is equivalent to the PRL species for  $Al_m$  being  $Al(OH)^{2+}$  instead of  $Al^{3+}$  or  $Al(OH)_3^0$ .

The difference between calculated and measured Gran ANC values increases as organic-acid concentration, reflected by DOC, increases. This is evident in surface-water data for Meander Lake basin in northern Minnesota (Fig. 3). Meander Lake DOC values average only about  $400 \mu\text{mol C l}^{-1}$ , but one of the lake's inlets drains a spruce bog and contains DOC values as high as  $2,400 \mu\text{mol C l}^{-1}$  (ref. 13). Concentrations of aluminium in Meander waters are very low (mean  $Al_m = 1.3 \mu\text{mol l}^{-1}$ ). The discrepancy between calculated and Gran ANC can be as large as  $50 \mu\text{eq l}^{-1}$  at DOC concentrations of  $800 \mu\text{mol C l}^{-1}$ . A single maximum discrepancy of  $180 \mu\text{eq l}^{-1}$  was observed for a sample with  $2,400 \mu\text{mol C l}^{-1}$ .

To explore the DOC-related discrepancy, Gran analyses were performed on theoretical titration data. The Gran analysis of ANC titration data requires plotting the Gran function  $F_1$  versus titrant volume.  $F_1$  is calculated as follows:

$$F_1 = (V + V_0)10^{-pH} \quad (5)$$

where  $V$  is the added titrant volume and  $V_0$  is the original sample volume. This produces a curve like that shown in Fig. 4a. The right-most 'linear' portion of the curve is extrapolated to determine the equivalent volume of titrant, and thus the ANC of the sample.

The Gran analysis procedure was applied to data generated by performing computer-simulated titrations using equilibrium constants associated with the carbonate system<sup>17</sup>. In addition, a variety of monoprotic weak acids with varying concentrations and fixed strengths ( $pK_A$  values) were added to the solutions, one at a time, to simulate the effects of organic acids ( $6.5 \mu\text{eq mg}^{-1}$  DOC, which is close to that observed by others<sup>25</sup>). The initial ANC of each solution was set to  $50 \mu\text{eq l}^{-1}$ , and 100 ml aliquots of the solution were titrated with 0.01 M strong acid, in 20  $\mu\text{l}$  increments to pH 3. Table 1 shows the results of the Gran plot analysis using points from pH 3.0 to 3.3 for the extrapolation.

These values suggest that the difference between theoretical and Gran ANC due to the organic acid is <5%, at organic-acid

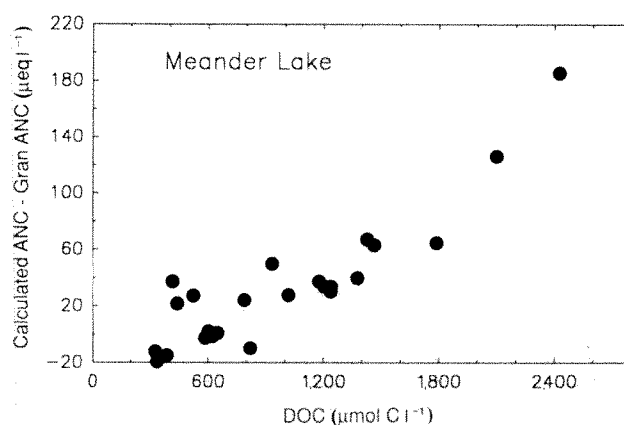


FIG. 3 The difference between calculated ANC (with 2 times  $Al_m$ ) and Gran ANC versus DOC for waters from the Meander Lake basin.



concentrations of  $0\text{--}25\ \mu\text{eq l}^{-1}$  ( $\sim 0\text{--}300\ \mu\text{mol C l}^{-1}$ ). At higher organic-acid concentrations the difference increases, reaching 28% at  $165\ \mu\text{eq l}^{-1}$  ( $2,100\ \mu\text{mol C l}^{-1}$ ). However, when these same synthetic titration data were analysed using a standard laboratory Gran analysis algorithm, the differences were larger. For an organic-acid concentration of  $25\ \mu\text{eq l}^{-1}$ , the difference was 10%, and it increased to 44% at  $165\ \mu\text{eq l}^{-1}$ .

The increasing curvature of the  $F_1$  function with increasing organic-acid concentration (Fig. 4b) can be problematic in analysis of titration data. The curve is nonlinear at much lower pH values (and thus higher  $F_1$  and  $V$  values) as the DOC concentration increases. If the titration is not carried out to a

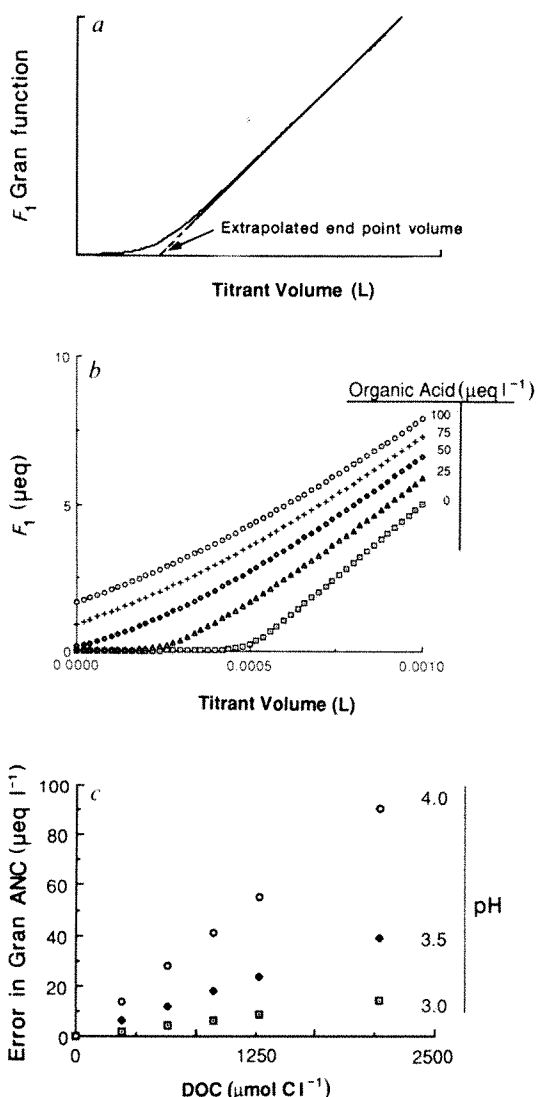


FIG. 4 Analysis of curvature in the Gran procedure: a, illustration of a Gran plot. The linear portion of the curve is extrapolated to determine the equivalent titrant volume. The slope of the line should correspond to the normality of the titrant. b, Curvature in  $F_1$  Gran function due to organic acids. Curves are for carbonate solutions to which varying amounts of organic acid ( $pK_A = 4.5$ ) have been added ( $ANC = 50\ \mu\text{eq l}^{-1}$ ). At higher organic-acid concentrations, the curvature becomes more pronounced, and the intercept with the titrant volume axis shifts to lower values. c, Error in Gran ANC due to curvature of the  $F_1$  function versus DOC, for different final titration pH values. Number of points in the  $F_1$  Gran function used to extrapolate were 35 out of 75 (pH 4.0), 90 out of 190 (pH 3.5), and 300 out of 600 (pH 3.0). Failure to titrate to low pH values can lead to large errors in ANC.

TABLE 1 Gran analysis

Initial ANC ( $\mu\text{eq l}^{-1}$ )	Organic-acid concentration ( $\mu\text{eq l}^{-1}$ )	Organic-acid strength $pK_A$	Gran ANC ( $\mu\text{eq l}^{-1}$ )
50	0	—	50
50	25	6.5	50
50	50	6.5	50
50	25	5.5	49.8
50	50	5.5	49.5
50	25	4.5	47.9
50	50	4.5	45.7
50	75	4.5	43.6
50	100	4.5	41.5
50	165	4.5	36.0

low enough pH, the curvature will not be minimized and thus ANC will be underestimated (Fig. 4c). As indicated, the error in Gran ANC can exceed  $90\ \mu\text{eq l}^{-1}$  for high DOC waters owing to curvature in the  $F_1$  function alone, if titrations are only carried out to pH 4.0. The error decreases as the final pH of the titration decreases. Below pH 3.0, however, the error associated with the precise measurement of  $H^+$  concentration becomes too large to further improve Gran estimates.

These calculations indicate that the curvature error can range from 0 to  $90\ \mu\text{eq l}^{-1}$  for organic-acid concentrations of  $0\text{--}165\ \mu\text{eq l}^{-1}$  ( $0\text{--}2,100\ \mu\text{mol C l}^{-1}$ ). The actual differences observed in the Meander Lake basin waters were considerably larger. We hypothesize that the additional differences result from increasing strength of the acid functional groups as the solution  $H^+$  concentration increases. This type of behaviour has been observed<sup>26</sup> and has been attributed to protonation of adjacent functional groups of polyprotic organic acids<sup>27</sup>.

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# Unexpected changes in the anoxic/anoxic interface in the Black Sea

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THE Black Sea is the largest anoxic marine basin in the world today<sup>1</sup>. Below the layer of oxygenated surface water, hydrogen sulphide builds up to concentrations as high as 425  $\mu\text{M}$  in the deep water down to a maximum depth of 2,200 m (ref. 2). The hydrographic regime is characterized by low-salinity surface water of river origin overlying high-salinity deep water of Mediterranean origin<sup>1,3</sup>. A steep pycnocline, centred at about 50 m is the primary physical barrier to mixing and is the origin of the stability of the anoxic (oxygen/hydrogen sulphide) interface. Here we report new observations, however, that indicate dramatic changes in the oceanographic characteristics of the anoxic interface of the Black Sea over decadal or shorter timescales. The anoxic, sulphide-containing interface has moved up in the water column since the last US cruises in 1969 and 1975. In addition, a suboxic zone overlays the sulphide-containing deep water. The expected overlap of oxygen and sulphide was not present. We believe that these observations result from horizontal mixing or flushing events that inject denser, saltier water into the relevant part of the water column. It is possible that man-made reduction in freshwater inflow into the Black Sea could cause these changes, although natural variability cannot be discounted.

The US-Turkish Oceanographic Expedition to the Black Sea consisted of five cruises on the RV *Knorr* from 15 April to 2 August 1988 (*Knorr* cruise 134, legs 8 to 12)<sup>4</sup>. One of the main goals was to study the anoxic interface in detail. The principal

features of the anoxic interface were summarized by Caspers<sup>1</sup>. Oxygen was found to be depleted to zero concentration at depths of between 125 and 300 m. During the RV *Atlantis II* cruise in March and April 1969 the boundary between the oxygenated surface waters and the sulphide-containing deep waters was found to occur along a constant-density horizon. An overlap of oxygen and sulphide of a few metres was observed<sup>2</sup>. The isopycnal surface (potential density,  $\sigma_\theta = 16.41$ ) of the oxygen zero boundary was dome-shaped and ranged from a depth of 115 m in the centre of the basin to about 275 m near the shelves<sup>3,5</sup>. The RV *Chain* visited the Black Sea in April 1975 and found hydrographic results that were nearly identical to those reported in 1969<sup>6</sup>. At that time the anoxic interface comprised a 20–30-m transition zone where oxygen and hydrogen sulphide coexisted at very low concentrations ( $<15 \mu\text{M}$ ) (ref. 7). Russian scientists have frequently reported the coexistence of oxygen and hydrogen sulphide in a zone called the 'C layer' (see, for example, ref. 8).

Several studies in the early 1980s<sup>8–12</sup> reported that the upper boundary of the sulphide zone was rising. We have confirmed such observations with high-quality continuous data and we find that the first appearance of sulphide in the water column is higher by as much as 30 m relative to the previous US studies in 1969 and 1975. In addition, we have also observed a significant gap of 10–40 m between the layer of oxygen decrease to suboxic levels (less than  $5 \mu\text{M}$ ) and that of the first appearance of hydrogen sulphide. This gap was thicker in the centre of the Black Sea than near its boundaries.

During the 1988 RV *Knorr* cruises we studied the chemistry and microbiology of the interface zone in detail using a new pump-profiling system. This pump sampler was attached to a CTD (conductivity, temperature and depth instrument) and interfaced to an autoanalyser for detailed analyses of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{Si}$ ,  $\text{PO}_4^{3-}$  and  $\text{H}_2\text{S}$ . The pump was stopped at specific depths to collect samples for manual analyses. The pump was lowered at a rate of 6–10  $\text{m min}^{-1}$  and data were acquired every 3 s, giving a sampling rate of 2–3 data points per metre. Under calm conditions, features in the water column could be resolved to better than 2 m. Within less than an hour the detailed structure of the water column was characterized on a down cast and a discrete sampling strategy designed for the subsequent up cast.

The potential temperature ( $\theta$ ) in the central part of the western basin of the Black Sea (station BS3-2;  $32^\circ\text{E}$ ,  $42^\circ50'\text{N}$ ; 5 June 1988)<sup>13</sup> decreased from high surface values of  $>17^\circ\text{C}$ , reflecting seasonal warming, to a minimum of  $6.95^\circ\text{C}$  at 45 m, then increasing to  $8.58^\circ\text{C}$  at 100 m and to  $8.9^\circ\text{C}$  at 2,050 m (Fig. 1a). Station 1445 of the 1969 *Atlantis II* Black Sea cruise<sup>2</sup> ( $31^\circ27'\text{E}$ ,  $43^\circ08'\text{N}$ ; 1 April 1969) was in close proximity to our station BS3-2 and

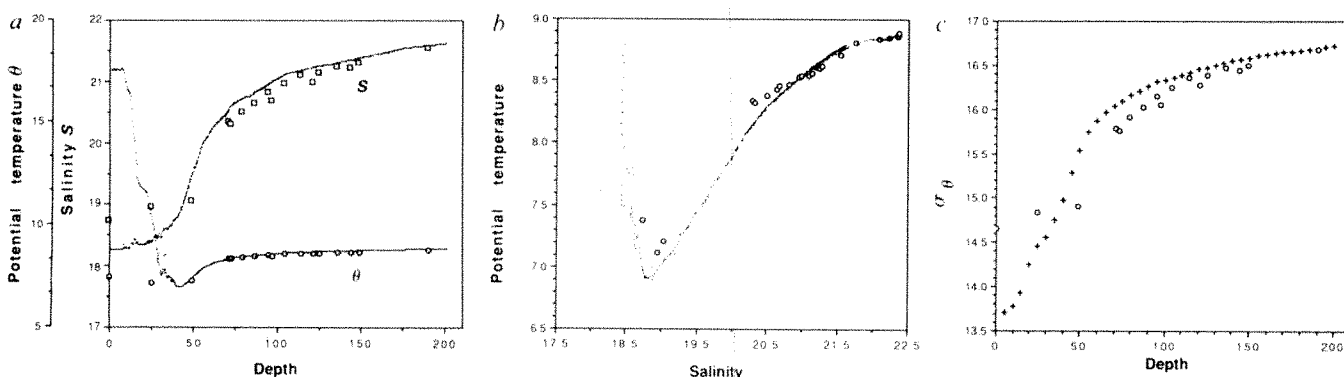


FIG. 1 Potential temperature, salinity and density in the Black Sea. Open symbols are from station 1445 of the March 1969 *Atlantis II* Black Sea cruise. Closed symbols are from station BS3-2 of cruise 3 of the US-Turkish expedition (June, 1988). a, Potential temperature ( $\theta$ ) and salinity (S) plotted

against water depth to 200 m, calculated from SeaBird CTD data with the assistance of G. White and M. Relander. b, Potential temperature against salinity for the entire water column. c, Potential density ( $\sigma_\theta$ ) against water depth to 200 m.

is used for comparison. The 1969 and 1988 data agree well in the range from 50 m and 100 m and below 200 m. From 100 m to 200 m the 1988 temperature is about 0.1 °C warmer. The surface temperatures are quite different because the cruises were made at different times of the year.

The salinity in 1988 increased from 18.33‰ at the surface to 21.09‰ at 100 m (Fig. 1a) and then to 22.33‰ at 2,050 m. The 1969 data are sparse above 70 m, but the salinity was definitely higher by at least 0.1‰ in 1988 over the 50–200 m depth range<sup>3</sup>.

The change in hydrographic properties can also be seen by comparing plots of the potential temperature versus salinity ( $\theta$ - $S$ ) (Fig. 1b). There is clearly a departure toward higher salinities at a given potential temperature for salinities greater than 21.00‰. The net result is that the potential density ( $\sigma_\theta$ ) was higher from 50 m to 200 m by up to 0.3 in 1988. This corresponds to an upward shift of the isopycnal surfaces in this depth interval by as much as 20 m. In 1969 the  $\theta$ - $S$  diagram was linear across the anoxic interface from 50 to ~400 m. In 1988 the  $\theta$ - $S$  data were linear from about 45 m to 70 m, and there was a small degree of curvature through the anoxic interface region. Since 1969 (and 1975) there has been a small but significant change in the hydrographic and density structure of the anoxic interface region (50–200 m) at this location in the Black Sea.

In comparison with the subtle changes in the water-column hydrographic and density structure, the changes in the oxygen, sulphide and nutrient distributions have been more pronounced. The new data show that dissolved oxygen is high in the surface water with a photosynthetically produced maximum at 10 m (Fig. 2a). Oxygen concentration decreases rapidly, to values of less than 5  $\mu$ M at a depth of 55 m. In 1969 the measured oxygen concentration did not decrease to zero until about 125 m depth. Sulphide first appeared at about 95 m and increased almost linearly with depth (Fig. 2a). Repeat casts indicated that the uncertainty in this depth was about 5 m. This first appearance of sulphide occurred close to the density surface of  $\sigma_\theta = 16.32$ . In 1969 sulphide first appeared at 125 m, corresponding to the density surface of  $\sigma_\theta = 16.39$ . Thus sulphide appears to have shoaled slightly relative to the water-column density structure.

A prominent new feature in the anoxic interface is a suboxic zone that exists from 55 m to 95 m at station BS3-2. This zone is characterized by oxygen concentrations less than 5  $\mu$ M, with no discernible gradients. This suboxic zone was found at all stations occupied during the 1988 expedition and was unexpected, because most recent literature<sup>3,14,15</sup> reported the coexistence of  $O_2$  and  $H_2S$ ; the vertical extent of this coexistence

was reported to be as large as 90–100 m (ref. 15) and appeared to vary laterally and seasonally<sup>8</sup>. There is one earlier report of a suboxic zone but it appeared to be limited to an isolated eddy experiencing unusually intense vertical mixing<sup>16</sup>.

Ammonia content (Fig. 2b) increased rapidly and linearly with depth below 80 m. The gradient relative to depth for ammonia in 1988 was similar to that in 1969<sup>2</sup>, but was shifted upward by about 50 m in the recent data.

The 1988 phosphate profile (Fig. 2c) is intriguing because it shows a maximum at 55 m, decreasing to a minimum at 70 m, and then increasing rapidly to a large maximum at 95 m, coincident with the first appearance of sulphide. Similar profiles were found at most offshore stations. Examination of the 1969 data suggests that two  $PO_4^{3-}$  maxima were present but could not always be identified unambiguously because of the coarse sampling resolution<sup>14</sup>. The spline fit of the total 1969 data set used by Shaffer<sup>17</sup> showed that they were probably present. The upper  $PO_4^{3-}$  maximum is probably due to release from decomposing organic matter during aerobic respiration<sup>14</sup>. The deeper  $PO_4^{3-}$  maximum probably results from reductive dissolution of solid Mn and Fe oxides, which release adsorbed  $PO_4^{3-}$  (ref. 17). Both  $PO_4^{3-}$  maxima appear to have risen in the water column by about 50 m relative to 1969.

The changes in the salinity and density structure may reflect horizontal mixing or flushing events that inject new water into the pycnocline. Several authors have proposed that the pycnocline is most probably influenced by lateral ventilation rather than by one-dimensional vertical transport (see, for example, ref. 18). The shallow temperature minimum bounded by the 8 °C isotherms is called the cold intermediate layer (CIL). At station BS3-2 the CIL is between 25 and 55 m. Tolmazin<sup>19</sup> has summarized the evidence (for example, ref. 20) to suggest that the water in the CIL originates in the Northwestern Shelf (NWS) region of the Black Sea. Here, the coldest temperatures and low river runoff in winter result in the formation of dense water which descends the slope and spreads across the rest of the Black Sea on a timescale of about one year. Ovchinnikov and Popov<sup>21</sup> have recently proposed the alternative view that the water in the CIL originates at the centres of the cyclonic gyres.

Regardless of the origin of the water in the CIL, its production rate and density must be closely linked to long-term variations in the freshwater and heat budgets of the Black Sea. When very dense water forms, the flow overshoots the traditional lower boundary of the CIL. Natural interannual or decadal variations in climate and river runoff can produce horizontal temperature and salinity (that is, density) waves in this shallow intermediate

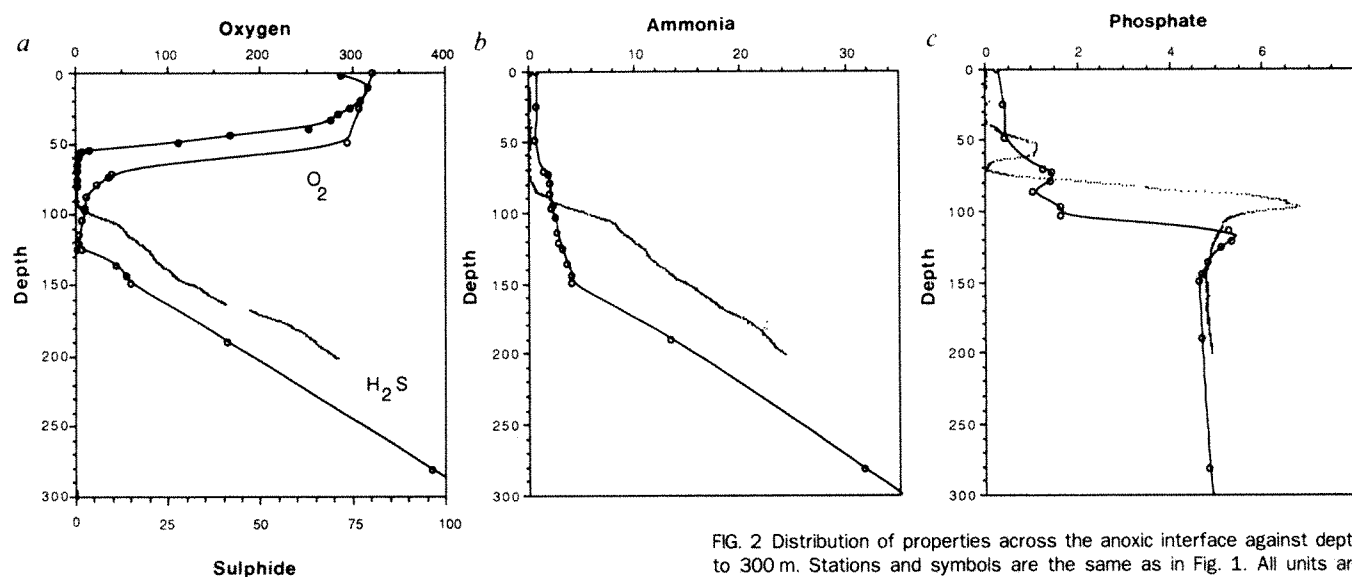


FIG. 2 Distribution of properties across the anoxic interface against depth to 300 m. Stations and symbols are the same as in Fig. 1. All units are  $\mu$ mol kg<sup>-1</sup>. a, Oxygen and hydrogen sulphide. b, Ammonia. c, Phosphate.



layer. Superimposed on these natural variations is the man-made reduction in the freshwater runoff from Soviet rivers entering the NWS region of the Black Sea. Tolmazin<sup>19,22</sup> reported that river flow of the Dnieper and Dniester had already been reduced by 50% in 1981. The total freshwater input from all rivers had been reduced by about 15%. Reduction in river input will also result in increased Mediterranean sea-water input through the Bosphorus<sup>23</sup>. Substantial reduction in freshwater influx should cause changes in the salinity structure of the upper layer of the Black Sea. The extreme case of no river input would eventually result in a Mediterranean-style circulation for the Black Sea.

Our data clearly show that the salinity and density in the depth range 50–200 m was high in 1988 relative to historical values. It is possible that a reduction in the freshwater inflow is resulting in the formation of intermediate waters that have higher salinities and densities than in the past. The data set is not complete enough, however, to allow us to distinguish man-made effects from possible natural variations.

Increased production of water to the shallow intermediate layers could be viewed as analogous to the mixing or flushing events that occur in other anoxic basins, such as Saanich Inlet<sup>24–26</sup> and the Baltic Sea<sup>27</sup>. These flushing events produce suboxic zones similar to the one recently observed in the Black Sea. Flushing is thought to displace anoxic water upward in the water column. The sulphide in this displaced water then reacts with oxygen to form an oxygen-depleted layer<sup>28</sup>. Any remaining oxygen is then consumed by aerobic respiration, thus producing a suboxic layer.

Additional chemical and microbiological results will need to be considered for a complete description of the changes in the

oxic/anoxic interface. The flushing-event hypothesis is attractive, however, because it can simultaneously explain the changes in salinity, density and concentration of oxygen, sulphide and nutrients. □

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## Static amorphization of anorthite at 300 K and comparison with diaplectic glass

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DIAPLECTIC glass, which is produced without fusion, is characteristic of meteorite impact sites in feldspar- and quartz-rich sites on the Earth and Moon; it has also been produced in laboratory shock experiments<sup>1–3</sup>. Its presence in nature is thus considered to be strong evidence of exposure to a high-pressure shock wave generated by impact. The formation of such glasses has been universally interpreted in terms of the brief, high-temperature and high-pressure conditions characteristic of shock loading. Here we report that crystalline anorthite ( $\text{CaAl}_2\text{Si}_2\text{O}_8$ ) becomes amorphous at static pressures of between 22 and 28 GPa at 300 K. The amorphization is associated with a pressure-induced increase in the coordination of silicon and aluminium by oxygen, from fourfold to five- and sixfold. The increase in coordination appears as the sample vitrifies at pressure; on decompression, the coordination reverts to fourfold. The vitrification is spatially heterogeneous, and may be crystallographically controlled. The formation of glass from crystalline material under static conditions at 300 K indicates that the high strain rates and temperatures associated with shock compression are not required for the creation of glasses without fusion.

The starting material used in this study is 'transitional' anorthite from Miyake-zima, Japan, and is identical to the sample material used in previous shock experiments<sup>4</sup>. Single crystals of this material, each about 15  $\mu\text{m}$  in thickness, were

statically compressed in a Mao-Bell-type diamond cell; pressures were determined using ruby fluorescence<sup>5</sup>. A 16:3:1 mixture of methanol, ethanol and water was used as a pressure-transmitting medium, as this mix is hydrostatic to pressures of 15 GPa (ref. 6). At higher pressures, the samples were sufficiently small that the pressure gradients across them did not exceed 1 GPa. In particular, samples never bridged the gap between the diamond anvils. Pressures were increased over about 10 minutes, and the samples were then held at each pressure for approximately 18 hours, which is about 11 orders of magnitude longer than the corresponding periods in shock experiments. High-pressure infrared spectra were measured using a technique described elsewhere<sup>7</sup>, and indices of refraction were measured by the oil-immersion technique at ambient conditions.

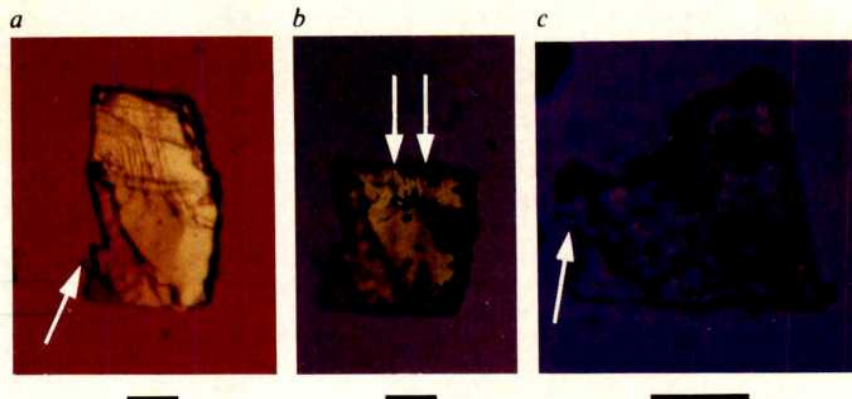
Figure 1a shows a single crystal of anorthite before compression. The sample is optically anisotropic, with a birefringence of 0.013, in accord with the typical value for such crystals<sup>8</sup>. Samples compressed to 15 GPa exhibit no evidence for vitrification, nor any departure from single-crystal characteristics. After compression to 22 GPa, however, two optically isotropic bands appear in the sample (Fig. 1b). Each of these is about 20  $\mu\text{m}$  wide, and they have a separation of about 40  $\mu\text{m}$ . The birefringence of the crystalline material is indistinguishable (to within 20%) from that of the starting material. After compression to 28 GPa, the sample is optically isotropic, with a birefringence of less than 0.002 (Fig. 1c). Thus, crystalline anorthite is transformed to a glassy state merely by static compression to pressures exceeding 25 ( $\pm 3$ ) GPa, at ambient temperature.

The index of refraction of a quenched isotropic sample of anorthite glass is 1.62 ( $\pm 0.02$ ), significantly higher than the mean value of 1.58 observed for both anorthite crystal and shock-produced anorthite glass<sup>9</sup>. Thus, it seems that the amount of time over which a sample is compressed, and possibly the post-shock thermal regime, may profoundly affect the degree of densification within pressure-amorphized silicates. Comparisons between glasses formed by shocks of different duration might not, therefore, yield reliable estimates of peak shock pressures.

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FIG. 1 Anorthite sample at zero pressure (outside the diamond cell) photographed with cross-polarized transmitted white light passing through a gypsum plate: *a*, before compression, *b*, after compression to 22 GPa, and *c*, after compression to 28 GPa. The sample is approximately 15  $\mu\text{m}$  thick and each scale bar is 50  $\mu\text{m}$  long. The multiply compressed sample fractured between photographs, but it is shown in the same orientation and arrows indicate the same feature in *a* and *c*. The two arrows in *b* indicate the vertically oriented isotropic bands that formed at high pressure. Note that the colour of the transmitted light is identical to the background colour in *c*, indicating that the sample is isotropic, except where small grains of ruby (used for pressure calibration) lie on its surface.



To determine the mechanism of the vitrification process, one must examine the microscopic structure of the sample as it amorphizes. Pressure-induced amorphization of two polymorphs of  $\text{SiO}_2$ , quartz and coesite, has been documented by Raman spectroscopy and X-ray diffraction<sup>10,11</sup>. As this amorphization involves the disappearance of both the X-ray diffraction pattern and, at higher pressures, the Raman spectrum, little information was obtained about the molecular-scale structure of the high-pressure amorphous phase. In contrast, infrared absorption spectra may characterize anorthite both before and after amorphization.

Figure 2 shows infrared spectra of initially crystalline anorthite as it is compressed to 1.7 GPa and 33.4 GPa, and then decompressed to 1.0 GPa. The strong absorption bands between 850 and 1,200  $\text{cm}^{-1}$  in the low-pressure spectra of anorthite are due to antisymmetric stretching vibrations of both the  $\text{SiO}_4$  and the  $\text{AlO}_4$  tetrahedra<sup>12</sup>. The intensity of these tetrahedral bands is decreased significantly in the spectrum of anorthite at 33.4 GPa, and there is an increased amplitude in the spectral region between 700 and 900  $\text{cm}^{-1}$ . Although anorthite undergoes a symmetry-lowering phase transition at 2.7 GPa (ref. 13), the effect of this structural change on the vibrational spectrum is small (Q.W. and R.J., unpublished results). Absorption between 700 and 900  $\text{cm}^{-1}$  is characteristic of silicon-oxygen and aluminium-oxygen polyhedra with octahedral and distorted octahedral coordination<sup>7,14</sup>. At 33.4 GPa, the broadening of the bands and the appearance of new bands in the spectrum, combined with our optical microscopic studies, indicate that the sample has become amorphous at this pressure<sup>15</sup>. In the spectrum of decompressed anorthite, the tetrahedral peaks have returned to their initial amplitude but the entire spectrum is broader and more poorly resolved than that of the starting material. This demonstrates, in accord with our optical observations, that the quenched sample is amorphous. Thus, the initially fourfold-coordinated crystalline anorthite is transformed under pressure to a glass with an increased coordination of aluminium and silicon. This densified, highly coordinated glass subsequently reconverts to a tetrahedrally coordinated glass on decompression. Such reversible transitions from fourfold to sixfold coordination of silicon and aluminium have been observed at 300 K within a glass of anorthite composition at pressures above  $\sim 11$  GPa (refs 7, 15).

Four mechanisms have been proposed previously for the creation of diaplectic glass: (1) reversion from a crystalline high-pressure phase on pressure release, (2) localized melting in 'shear bands', (3) defect-mediated amorphization, and (4) formation of a high-density glass under pressure. The first of these presumes that a crystalline high-pressure phase is formed under shock, which then reverts to glass during the high-temperature release from the shocked state. This model was proposed primarily because the density of framework silicates is large under shock compression, and it explicitly assumes thermodynamic equilibrium in the shocked state<sup>16</sup>. For com-

parison, the equilibrium crystalline assemblage of anorthite composition at pressures above 15 GPa is a three-phase mixture of  $\text{CaSiO}_3$ -perovskite, corundum and stishovite<sup>17</sup>. The amorphization that we observe is completely distinct from a transition to these phases, however. Moreover, corundum and stishovite are quenchable down to 1 bar pressure, yet are not present in our decompressed samples. Thus, we conclude that the transition to an amorphous state occurs under pressure as a non-equilibrium, kinetically hindered process.

Formation of diaplectic glass by local melting in shear zones—regions of high temperature created by intense deformation at high strain rates—has been proposed on the basis that shock deformation is spatially heterogeneous<sup>9,18–21</sup>. Both theoretical and empirical results support the shear-band model of shock deformation, but no correlation with glass formation has been demonstrated. Because neither high strain rates nor high temperatures are involved in glass formation in these experiments, we conclude that neither of the first two models, post-shock reversion of a high-pressure crystalline phase and localized melting in shear bands, satisfactorily explains the formation of pressure-induced glasses.

The last two mechanisms—defect-mediated processes (perhaps involving high dislocation densities) and direct conversion of low-pressure framework silicates to denser glasses under pressure—are both plausible means of amorphization<sup>3,22–24</sup>. These mechanisms of glass formation are not mutually exclusive, and they are supported by our observations. For example, the infrared spectra indicate that the coordination of aluminium and silicon increases at high pressures; the more highly coordinated species must, when present in small concentrations, constitute defects in the feldspar structure. Neither of these mechan-

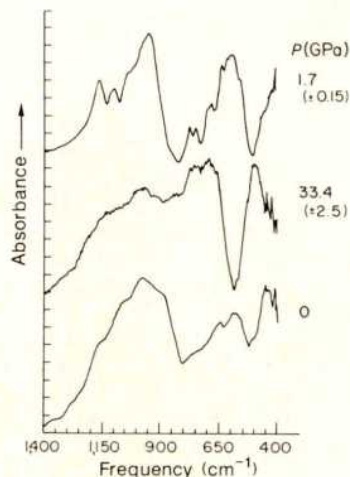


FIG. 2 Infrared absorption spectra of anorthite at 1.7 GPa (top), at 33.4 GPa (middle), and on subsequent decompression to 1.0 GPa (bottom), all at 300 K.



isms, however, predicts explicitly the spatially abrupt transition that we observe between crystal and glass<sup>21-25</sup>. If defect generation (or increases in coordination) were homogeneous within the sample, amorphization would be expected to constitute a macroscopically uniform transformation. In this context, our observation that the amorphous regions are initially confined to sharp bands within the crystalline material affords an interesting comparison with shock-generated diaplectic glasses (Fig. 1b). Anorthite samples recovered from shock pressures of 30 GPa contain planar, crystallographically oriented lamellae of glass, juxtaposed with crystalline anorthite<sup>25</sup>. Although the features observed in the shock-compressed samples are typically of sub-micrometre size, the planar features observed in our sample may be of similar origin. We speculate that, following the deformation of a tetrahedron or the collapse of a ring of tetrahedra to more highly coordinated species, subsequent deformations are controlled crystallographically and nucleate preferentially near the initial defect.

The mechanism proposed here by which crystalline feldspars are converted, via an amorphous, highly coordinated structure, to a glass provides a plausible explanation for the lack of significant argon loss from feldspars during the shock-loading process<sup>26,27</sup>. Such loss has been reported when the sample melts under shock, as is expected in the shear-band model of diaplectic-glass generation, or at pressures above about 50 GPa, for which melting of feldspars is inferred to occur<sup>23,24</sup>. In the formation mechanism proposed here, the coordination of the glass changes but there is little diffusional motion, and the transformation proceeds entirely in the solid state. Thus, we would expect shock-loading itself to induce little argon loss in feldspars at pressures under 50 GPa, although high post-shock temperatures may cause significant loss at lower pressures<sup>26</sup>.

In the case of SiO<sub>2</sub>, the properties of glassy and crystalline silica are such that the free energies of the ordered and disordered phases become similar at the pressure at which amorphization occurs. Thus, it has been proposed that the transition from high-pressure crystal to amorphous solid is described quantitatively by (metastable) equilibrium thermodynamics<sup>11</sup>, with kinetic effects preventing formation of the stable crystalline phase(s). An analysis of the relative stabilities of anorthite and anorthite glass is consistent with our observations: using available elastic and thermodynamic properties<sup>13,28-30</sup> and assuming a value of 4 for the derivative of the bulk modulus with respect to pressure for each phase, we predict a pressure of 28 GPa for the crystal-to-amorphous transition in CaAl<sub>2</sub>Si<sub>2</sub>O<sub>8</sub>. Because the elastic properties of anorthite glass are poorly constrained<sup>29</sup>, however, the agreement of this prediction with our observations may be fortuitous. Moreover, it is possible that non-hydrostatic stresses play a role in determining the pressure and temperature range, and perhaps even the occurrence, of this crystal-to-glass transition. We cannot preclude this possibility, but are unable to resolve such effects on the amorphization pressure. As the shocked state is also heterogeneous, with local regions of high differential strain<sup>19</sup>, we conclude that the different states of stress and strain rate in shocked and statically compressed samples are not significant in the formation of diaplectic glass.

The inferred coordination changes in these metastably compressed silicates probably involve significant density increases. Large increases in density are observed on the Hugoniot shock-compression curve at those pressures at which diaplectic glass is formed<sup>16,31</sup>. We therefore attribute the observation of a densified, mixed-phase regime along the Hugoniot of silicates<sup>16,31,32</sup> to the presence not of coexisting low- and high-pressure crystalline phases, but of a mixed-coordination glass created by shock-loading. This is supported by the extreme difficulty in quenching crystalline high-pressure phases from Hugoniot conditions<sup>33,34</sup>.

The presence of diaplectic glass in natural samples has long been interpreted as field evidence for impact-induced shock-loading. Although our results show that diaplectic-like glass can

be formed statically, we emphasize that the high pressures and relatively low temperatures at which this transition takes place are unlikely to be brought about by any known tectonic or volcanic process, whether in subduction zones<sup>35</sup> or explosive eruptions<sup>36</sup>. Thus it remains probable that such glasses are formed naturally only by impact-induced shock compression.

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## Carbonate melt from the mantle in the volcanoes of south-east Zambia

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RECENTLY Wallace and Green<sup>1</sup> reported the experimental formation of a dolomitic (Ca-Mg) carbonate melt in equilibrium with peridotite minerals in the range 21-30 kbar and 930-1,080 °C. These results confirm earlier deductions that, in the presence of CO<sub>2</sub>, initial melts from the mantle would be carbonatitic<sup>2,3</sup>, and extend the conditions of carbonate melt generation. The dolomitic melt composition also endorses earlier observations of quench dolomite after experimental melting of a natural garnet peridotite<sup>4</sup>. Dolomitic ashes, erupted from volcanoes near the confluence of the Rufunsa and Luangwa rivers in south-east Zambia<sup>5,6</sup>, offer the nearest analogue to the experimental melt yet reported. Quenched melt droplets in the volcanics now reveal new evidence indicating a mantle source for this natural dolomite liquid. Specifically, I present here results which show that the liquid contains magnesiochromite crystals (52% Cr<sub>2</sub>O<sub>3</sub>) that match those in mantle peridotites, kimberlites and lamproites. In contrast with the experimental liquid, the natural dolomitic melt has a low iron content,

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and high manganese and strontium, with alkalis virtually absent. High potassium activity is recorded, however, in the intensely metasomatized rocks around the main volcanoes. These differences suggest that the mantle source region chemistry differs from the high-sodium source envisaged in the experiments. The Zambian carbonatites thus reveal new aspects of carbonate melt and fluid activity in the Earth's mantle.

Volcanism in the Rufunsa region was approximately contemporaneous with the Chilwa Alkaline Province in Malawi (early Cretaceous, 135–105 Myr ago)<sup>7</sup>, and during the activity the three major centres were intruded by coarse-grained carbonatites, similar in all essentials to the classic intrusive complexes of Chilwa and elsewhere<sup>8</sup>. At Rufunsa, however, the intrusions were pierced by eruptions of pyroclastic dolomitic carbonatite unique to this locality. Sub-aerial volcanics of the same composition are preserved around the two southernmost complexes. Widely scattered smaller vents, ranging in size down to a few metres across, are filled solely with dolomitic tuffsite which is in most cases the product of a single eruptive episode. All the volcanological features indicate diatreme eruption through the crust by high-velocity, particle-charged streams of melt or fluid. Alkaline silicate magmatism (which normally accompanies carbonatite activity) is absent from the Rufunsa volcanism; this too is consistent with eruption directly from the site of melt formation. Activity was clearly localized by the intersection of the Luangwa and lower Zambezi rifts (Fig. 1). Rift intersections provide favourable sites for alkaline and carbonatite magmatism in continental interiors<sup>9,10</sup>, where the complex stress field favours perforation of the crust by deep-seated magmatism. Both the volcanology and the tectonics therefore point to high-speed eruption of the Rufunsa carbonatite volcanics directly from the mantle<sup>5,6</sup>. Eruption speeds for CO<sub>2</sub>-rich (diatreme-forming) melts from the mantle have been calculated by different methods<sup>11–13</sup>, and values of around 70 km h<sup>-1</sup> are 'generally regarded as reasonable'<sup>12</sup>. Solids would be entrained in a melt,

or fluid, or in melt + fluid, the appearance of fluid being strongly system-dependent<sup>14</sup>.

A striking example of the carbonatite tuffsite is preserved as one of the final eruptions through the axial zone of the Chasweta volcano. The rock is a mixture of angular accidental fragments (from the vent walls) and clear, rounded lapillae, in a dark red, fine-grained matrix (Fig. 2). Some lapillae have droplet shapes, and similar clear material also appears in intricate curved wisps and crescentic forms. Many lapillae enclose accidental fragments, typically of barite, coarse carbonate or feldspar. As with the other lapillae, the enclosing jackets consist mainly of very fine-grained colourless carbonate, with textures ranging from interlocking granular to trachytic. Deposition of carbonate melt on high-level accidental fragments is consistent with the proposal that separation of carbonate melt and fluid may occur only as the eruption nears the surface<sup>14</sup>. At this point, separated melt could be quenched almost instantaneously in supercooled fluid, which would explain the preservation of fragile melt wisps. The quenched melt is largely Mn- and Sr-rich dolomite (Table 1, column 3), whereas the surrounding matrix is iron-oxide-rich, implying that iron was strongly segregated into the transporting fluid phase. In most of the carbonate analyses, alkalis were either not detected or were present close to the detection limit.

Probably the most remarkable finding in the microprobe examination of the carbonate lapillae was the presence of chrome-rich spinel. The same mineral is found also in the intrusive tuffsites of Mwambuto, the major volcanic centre to the west of Chasweta. The spinel is dispersed as minute octahedra (<100 µm) through the carbonate; its distribution, euhedral form and the strong chemical zoning (Table 1, columns 4 and 5) indicate crystallization from the carbonate melt, probably on the liquidus. It has generally been accepted that 'carbonatites are not chromium-rich'<sup>15</sup>; one published average<sup>16</sup>, for example, gives a Cr content of 66 p.p.m., compared with 117 p.p.m. for average igneous rock. (Rufunsa melt lapillae have an estimated Cr content of around 1,000 p.p.m.) The Rufunsa volcanics are the first recorded instance of magnesiochromite-bearing carbonatite melt. For carbonatites worldwide, the spinel always hitherto reported is magnetite<sup>17</sup> (which has a very low Cr content). At Rufunsa, magnetite is common in the coarse-grained intrusive carbonatites, emphasizing that the magnesiochromite-bearing pyroclasts are not simply volcanoclastic equivalents of normal carbonatite but must have a different origin. Chrome-rich spinels of this composition are typically associated with ultramafic rocks of high-pressure origin, the compositions in Table 1 matching those reported from mantle xenoliths in kimberlites<sup>18,19</sup>. The compositions found are the same as early-crystallizing spinels both in kimberlites them-

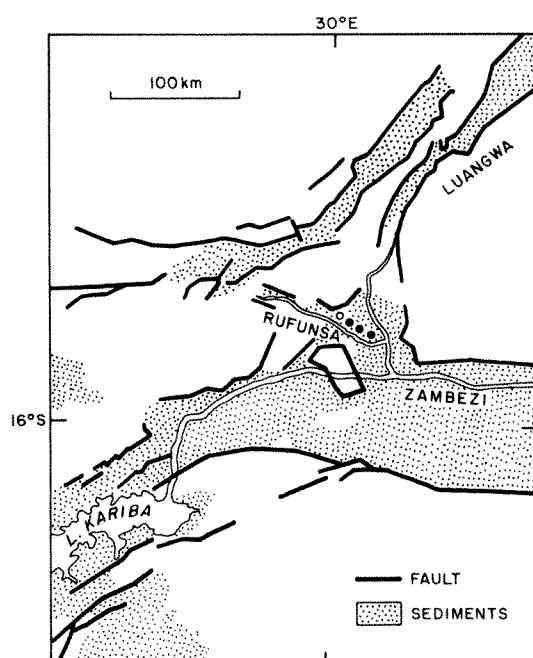


FIG. 1 Sketch map showing the intersection of the Luangwa (north-east-south-west) and Lower Zambezi Rift zones (rift depressions largely marked by sediments younger than 208 Myr (stipple), with older crystalline rocks (blank) forming higher ground); based on sheet 5 of ref. 29. The three main Rufunsa carbonatite volcanoes are indicated by filled circles. Sample H.780 comes from the vent of the most south-easterly volcano, Chasweta. The confluence of the Luangwa and Zambezi rivers marks the common border point of Zambia, Zimbabwe and Mozambique.

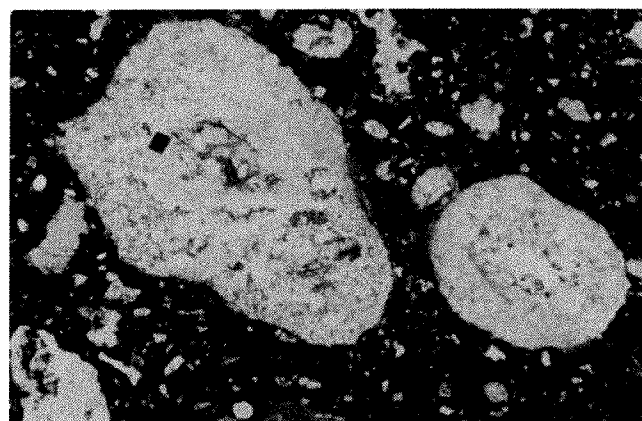


FIG. 2 Photograph of a thin section of the carbonatite volcanic in the vent of the Chasweta volcano. The rounded clear fragments are chilled carbonate melt droplets. Field of view is 3.4 mm wide.



selves<sup>20</sup> and in the 'group 1' spinels of lamproites<sup>21</sup> (both magma types may be diamondiferous and hence of mantle origin). Two other characteristics of the Rufunsa spinels confirm their high-pressure source. Haggerty<sup>19</sup> points out that low-pressure spinels are marked by higher levels of Ti and Fe<sup>3+</sup>, both of which are very low in the Rufunsa spinels. More importantly, he notes that spinels from the deepest mantle samples show a trend of falling Cr and rising Al with decreasing pressure and temperature, which is precisely the trend from core to rim in Rufunsa spinels. Rapid ascent of the Rufunsa melt, carrying the earliest-formed spinels, would provide such conditions of falling pressure and temperature, which are now recorded in the outer zones of the crystals by falling Cr and rising Al.

The association of carbonatites with mantle-derived magmas such as nephelinites, melilitites and kimberlites (which themselves may carry mantle xenoliths) puts their ultimate mantle source beyond question. In the Rufunsa province it is deduced, on the following grounds, that the carbonatite pyroclasts were erupted directly from the mantle source: (1) Rift intersection tectonics and diatremic style of eruption are consistent with direct tapping of the mantle<sup>5,6</sup>; (2) The dolomitic composition is that predicted by experiment for carbonate melts from a high-pressure peridotite source<sup>1,4</sup>; (3) The presence of magnesiochromite emphasizes the ultramafic connection; (4) Magnesiochromite compositions completely match those of high-pressure mantle rocks and accepted mantle melts (kimberlites/lamproites); (5) Zoning in the magnesiochromite is consistent with crystallization under conditions of falling *P* and *T* during magma ascent from the mantle source.

Attention has been drawn previously to the lack of sodium minerals in the Rufunsa volcanism<sup>6,22</sup>, the only alkali silicates in these volcanics being the K-Mg mica, phlogopite, and pure potash feldspar<sup>6</sup> (Table 1, columns 7 and 8). Both minerals are contained in fragments of metasomatized wall rocks. Phlogopite is very abundant in some eruptives, pointing to a greater abundance at depth and to the likelihood that the carbonatite melt source would have had phlogopite, rather than amphibole, as the major alkali-bearing phase. Experiments show that Mg-

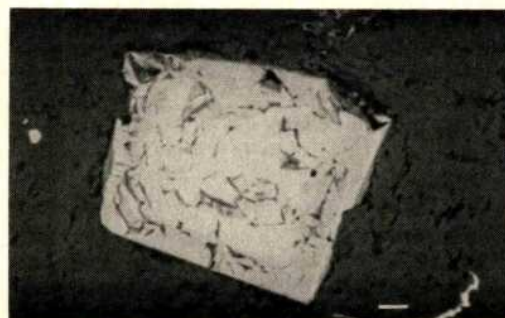


FIG. 3 Scanning electron microscope (back-scatter) image of a polished section of sample H.780, showing euhedral, zoned magnesiochromite in a carbonate matrix (analyses in Table 2). Scale bar, 10  $\mu$ m.

bearing carbonate melts must be expected from a phlogopite-bearing mantle<sup>23,24</sup> at depths below the amphibole stability region<sup>1</sup>, but analysis of the minute near-solidus liquids has not yet been possible.

For a more complete picture of the magmatic chemistry one must combine the melt composition, as revealed in the lapillae, with available data on the fluids in the system. In the dark red matrix of the volcanics, the iron content (as Fe<sub>2</sub>O<sub>3</sub>) may be as high as 10%, which must be ascribed largely to the transporting fluid, as no known country rock could provide an adequate source by contamination. Around the main vents, where there was sustained activity, potassium metasomatism affects all wall rocks regardless of initial composition, and its ultimate source must be in the magma system. Late fluids rich in Ba, P, S and F also cut all earlier rocks in the volcanoes. The total primary carbonatite budget should therefore include Mg, Ca, Mn, Sr, CO<sub>2</sub>, P, Al, Cr and Fe in the melt, and K, Fe, Ba, S, P and F partitioned into fluids at lower pressure. Of these, only Mg and Cr (and possibly Al) would be expected in higher concentrations in the source mantle; all the other elements must be regarded as strongly partitioned from the source mantle into the carbonatite. Separation of melt and fluid at low pressures means that only the broad attributes of the total chemistry may be accessible from the volcanic system, but even this initial assessment reveals some remarkable parallels with melt inclusions in diamonds<sup>25</sup>. Most notable in this respect is the balance of Ca and Mg, and the high levels of K, Fe, Ba and Sr in the diamond inclusions. Clearly, the Rufunsa carbonatites point the way to a new understanding of melt/fluid processes in the mantle.

There are many links between carbonatites and kimberlites, and in a few cases there are records of the presence of diamonds in the carbonatite association (refs 26, 27 and M. S. Garson, personal communication). It should be noted that kimberlites (of uncertain age) are also found along the Luangwa rift zone<sup>28</sup>, some about 400 km to the south-west in Zimbabwe and some (diamondiferous) about 400 km to the north-east in Zambia. Typical indicator minerals of kimberlite, such as picro-ilmenite and pyrope, were not sought in the original survey of Rufunsa, when the chief economic targets were Nb and P mineralization. In view of the mantle origin of the carbonatite volcanics, however, the possibility cannot be excluded that the Rufunsa volcanics may themselves contain diamonds. □

TABLE 1 Carbonatite volcanic rock (H.780) and constituent minerals compared with experimental run products (\*) at 22 kbar pressure and 1,000 °C

	1	2*	3	4	5	6*	7	8
SiO <sub>2</sub>	—	2.94	—	—	—	—	64.79	42.02
TiO <sub>2</sub>	—	0.45	—	1.29	1.78	0.61	—	0.97
Al <sub>2</sub> O <sub>3</sub>	—	1.95	—	13.48	28.65	58.53	17.37	10.37
Cr <sub>2</sub> O <sub>3</sub>	—	0.22	0.26	52.68	34.47	8.02	—	0.12
FeO	0.82	4.61	0.49	18.84	19.67	29.75	0.38	9.82
MnO	1.61	—	1.56	—	—	—	—	0.39
MgO	18.72	14.19	19.00	11.94	14.19	13.89	—	19.65
CaO	31.84	21.29	28.84	—	0.37	0.18	0.33	—
K <sub>2</sub> O	—	0.35	—	—	—	—	16.40	10.63
Na <sub>2</sub> O	—	4.99	—	—	—	—	tr.	0.34
P <sub>2</sub> O <sub>5</sub>	0.45	0.48	—	—	—	—	—	—
SrO	—	—	1.10	—	—	—	—	—
BaO	—	—	—	—	—	—	0.32	0.22
Total	53.44	51.49	51.25	98.23	99.13	100.00	99.59	94.53

(1) CaO, MgO and MnO as carbonate in whole rock H.780 (ref. 5). Individual mineral analyses indicate small amounts of iron carbonate (compare column 3), and a mean value for FeO (~0.5 MnO) is applied here.

(2) Experimental carbonate melt in equilibrium with mantle silicates (Table 1, column 3 of ref. 1).

(3) Melt carbonate grain adjacent to chromian spinel (column 5), H.780.

(4) Magnesiochromite (core of crystal) in carbonate lapillus (Fig. 3).

(5) Chromian spinel (rim) in carbonate lapillus.

(6) Spinel in equilibrium with experimental carbonate melt (Table 1, column 9 of ref. 1). (Note that the spinel is aluminous, as expected for ~22 kbar pressure, in contrast with magnesiochromite typical of higher pressures<sup>19</sup>.)

(7) Potassium feldspar fragment in H.780.

(8) Phlogopite fragment in H.780.

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## Mate guarding as paternity insurance in Idaho ground squirrels

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FOLLOWING a copulation, males in many species of vertebrates (particularly birds)<sup>1-4</sup> and invertebrates<sup>5,6</sup> remain near the inseminated female and repel other suitors with displays or force. Guarding males must delay resumption of competitive mate searching<sup>7</sup>, but they may insure their paternity by reducing possibilities for secondary matings and sperm competition<sup>8,9</sup>. Among mammals, post-copulatory mate guarding has been reported in rodents<sup>10-12</sup>, mongooses<sup>13</sup>, ungulates<sup>14,15</sup> and primates<sup>16,17</sup>, including humans<sup>18</sup>, but the effects of such behaviour on male reproductive success have not been determined genetically. I report here that mate guarding by male Idaho ground squirrels (*Spermophilus brunneus*) enhances a male's probability of paternity. Furthermore, an analysis based on game theory shows that mate guarding is an evolutionarily stable strategy for male *S. brunneus*, but not male Belding's ground squirrels (*S. beldingi*), which resume searching once copulation is completed.

The Idaho ground squirrel is the rarest and least known of the 18 North American *Spermophilus* species. It is endemic to an area of only ~8,000 km<sup>2</sup> in west-central Idaho, and the population is divided into small demes (many with <300 animals)<sup>19</sup>. During 1987 and 1988 I studied a deme of ~100 individuals located in a short-grass meadow at 1,870-m elevation (45°00' N, 116°40' W), 34 km northwest of Council, Idaho. Ground squirrels there are diurnally active for just over 4 months in the year, emerging from hibernation in late March and disappearing again by early August.

Every ground squirrel in the study population was marked with black hair dye for visual recognition and with eartags for permanent identification. The sexual behaviour of ground squirrels was observed on 13 days in 1987 (between 31 March and 14 April) and on 12 days in 1988 (4-17 April), for a total of 228 man-hours. Females were sexually attractive to males for only a few hours on the first or second afternoon following their emergence from hibernation. Newly emerged females remained near their hibernacula, where they were located and courted by adult males (≥2 years old), who themselves had emerged 1-2 weeks previously.

Copulations occurred below ground and so were not observable. Matings were inferred when four criteria were met<sup>20</sup>: male (1) followed a female closely and sniffed or licked her genitalia, then (2) accompanied her into a burrow, where (3) the pair remained for >5 min, after which (4) a sperm plug was observed in the female's vagina<sup>21</sup>. These criteria were fulfilled for each female on only one afternoon in the year, and pup invariably appeared above ground 50-52 days later. Thus I am confident that a female's oestrus and her copulatory behaviour were appropriately interpreted.

By watching continuously I recorded all male attendants to and presumed copulations of, 12 females in 1987 and 14 female in 1988. Following matings, males stayed close to females: male was ≤1 m from each focal female in 87% ± 15% (s.d.) of scan samples taken every 30-45 min after a female's first mating (total scans = 179). A given male attempted to keep his mate in a small area by herding, and he often followed her down burrow then immediately re-emerged and blocked the entrance with his body. If a guarding male temporarily lost contact with his female (for example, if she went into a burrow unobserved) he gave a staccato chirping vocalization until she was relocated

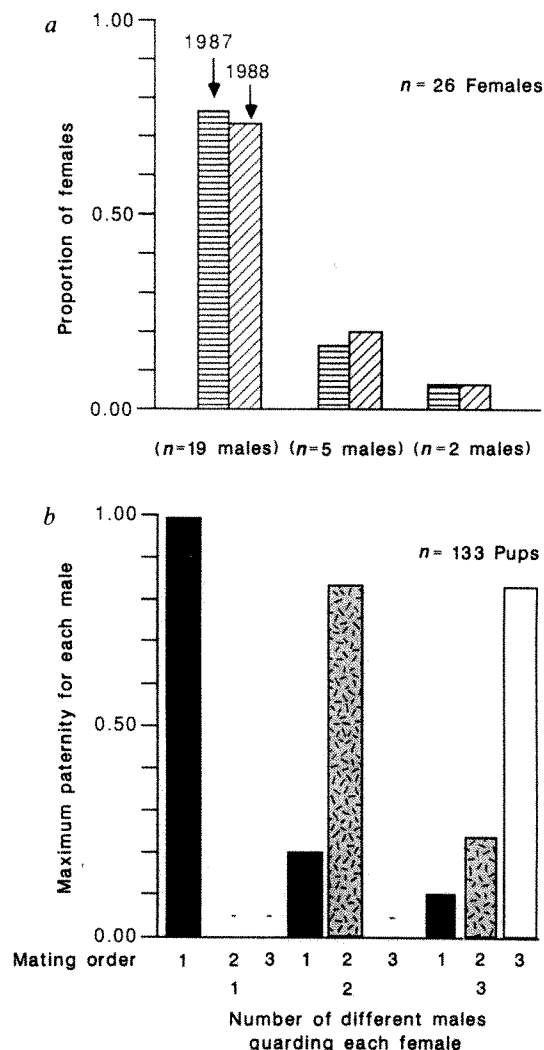


FIG. 1 *a*, The number of different males that guarded and apparently mated with 26 focal female Idaho ground squirrels (*S. brunneus*) in 1987 and 1988. *b*, Results of the electrophoretic paternity exclusion analyses, showing the highest proportion of the total offspring born to focal females that could have been sired by each successive guarding male (sample sizes are 97, 27 and 9 pups born to females guarded by 1, 2, and 3 males, respectively; data from 1987 and 1988 are combined).



the 'lost female' call was heard only in this context. Consort pairs went underground together every  $39 \pm 20$  min. and spent  $9.4 \pm 1.3$  min there. I captured 11 pairs the first time they resurfaced, and eight of the females had fresh vaginal plugs, indicating a recent copulation.

When a conspecific male approached a consort pair, the guarding male attacked him. In 72% of approaches ( $\leq 3$  m;  $n = 57$ ) the intruder fled, whereas in 28% he remained and fought. Usually males successfully sequestered their mate: of the 26 focal females, 19 were guarded by only one male and seven were guarded sequentially by multiple males (Fig. 1a). Guarding lasted  $2.9 \pm 1.1$  h regardless of the number of males involved, and terminated only when females were no longer approached by rival males (that is, behavioural oestrus was over). During the 1987-88 mating periods the average number of females guarded by each male was  $1.6 \pm 0.9$ , and the greatest number guarded successfully by any one male was four.

Successful guards had to repulse competitors  $4.3 \pm 1.7$  times during their female's oestrus. Although males whose takeover attempts were thwarted did not sustain observable injuries in fights, it took them  $1.3 \pm 0.5$  h ( $n = 27$ ) to locate (come within 3 m of) another oestrus female, because females were scarce and scattered (Table 1).

Nine males began guarding females, but were displaced. Seven of these were ousted during the first third of the female's period of behavioural oestrus. Subsequent guards were always heavier than the male they displaced, and there was a positive correlation between the length of guarding and male body weight ( $r = 0.79$ ,  $P < 0.01$ ). Within 20 min after a change of the guard, the new male accompanied the female underground, where they presumably copulated. Ousted males immediately resumed searching, but every receptive female approached by these males was already being guarded, and only one was successful in taking over a female. Additionally, two were carried off by hawks (one by a prairie falcon, *Falco mexicanus*, and the other by a goshawk, *Accipiter gentilis*).

The day after a female was guarded she began constructing a nest burrow and excluding all conspecifics, both males and females, from a small territory surrounding it. Females reared their litter of  $5.2 \pm 1.4$  young (range: 2-7,  $n = 59$  litters) alone, and males did not live near their mates or behave parentally.

To investigate the genetic consequences of mate guarding, I live-trapped all 26 focal females, the 20 different males that guarded them, and all 133 pups they weaned (69 in 1987, 64 in 1988). A small sample of blood ( $\sim 1$  ml) was pipetted from the suborbital sinus of each animal, separated by centrifuging, and stored in liquid nitrogen ( $-196^\circ\text{C}$ .) until analysis (8-10 weeks). Proteins were studied using standard horizontal starch-gel elec-

trophoresis and histochemical staining procedures<sup>22,23</sup>. Of 32 putative loci assayed, seven were polymorphic in blood (allele frequencies are in parentheses): adenosine deaminase (0.64, 0.36), peptidase with glycyl-leucine (0.60, 0.40), peptidase with leucyl-glycyl-glycine (0.92, 0.08), esterase A (0.84, 0.16), protein 1 (0.85, 0.14, 0.01), protein 2 (0.79, 0.21), and protein 3 (0.92, 0.08). For these allozymes, frequencies of homozygotes and heterozygotes did not differ from Hardy-Weinberg expectation (all  $P \geq 0.10$ ,  $\chi^2$  tests), and there was no evidence of linkage disequilibrium (all  $P \geq 0.20$ ,  $G$  tests) or sex-linkage.

To analyse paternity, the phenotype of every animal was determined for all seven variable loci. Then, for each litter, the phenotype of every juvenile and its dam was used to implicate or eliminate each guarding male as the sire. The results (Fig. 1b) indicate that pups born to females that had been guarded by only one male were indeed sired by that male. Litters of females guarded by more than one male were typically multiply sired (five of the seven). However, paternal representation was nonrandom: a minimum of 66-100% of pups in each of these seven litters were sired by the last/longest attending male. These results could be due either to a preponderance of sperm from the male that guarded each female longest (that is, the one that apparently mated most often) or to last male sperm precedence<sup>24,25</sup>. Preponderance is suggested by a nonsignificant but positive correlation between the number of (apparent) copulations by first guards and their maximum paternity among litters of multiply guarded females.

Male behaviour and paternity have been analysed in three other species of ground-dwelling sciurids. Black-tailed prairie dogs (*Cynomys ludovicianus*)<sup>26,27</sup> and yellow-bellied marmots (*Marmota flaviventris*)<sup>28,29</sup> are harem polygynous: an adult male lives with a group of females throughout the year and protects them from intrusions by conspecific males and predators. The resident male sires the vast majority of the young and he behaves parentally toward them. Belding's ground squirrels (*S. beldingi*), by contrast, are a lekking species: males aggregate near females' hibernacula to mate, but subsequently do not live near those females or behave parentally<sup>30-32</sup>.

In Belding's ground squirrels, receptive females were clustered (Table 1), and it took males only  $0.4 \pm 0.1$  h ( $n = 88$ ) after being defeated in a fight over a female to locate and initiate courtship with a new partner (significantly faster than male Idaho ground squirrels,  $P < 0.001$ , Mann-Whitney  $U$  test). Female *S. beldingi* typically mated with 3-5 partners, most litters were multiply sired<sup>33</sup>, and paternity varied with mating precedence: a female's first mate sired on average 0.6 of the litter, her second mate 0.3, and her third and subsequent mates 0.1 (unpublished data). Male *S. beldingi* did not guard females, but

TABLE 1 Comparison of the availability and localizability of sexually receptive females in Idaho ground squirrels and Belding's ground squirrels

Species (study site)	Year (length of mating period)	Oestrus females available per day* (range)	Distance between burrows of receptive females† (m)	Average operational sex ratio‡ (range)
<i>S. brunneus</i> (Council, Idaho)	1987 (13 days)	$1.4 \pm 1.1$ (0-4)	$128 \pm 39$ ( $N = 11$ female pairs)	1:3.2 (1:2-1:4)
	1988 (12 days)	$1.6 \pm 1.3$ (0-5)	$110 \pm 34$ ( $N = 13$ )	1:2.7 (1:2-1:4)
Significance level of comparison§		↑ ( $P < 0.01$ )	↑ ( $P < 0.001$ )	↑ NS
<i>S. beldingi</i> (Tioga Pass, California <sup>30</sup> )	1975 (16 days)	$2.6 \pm 1.2$ (1-6)	$10 \pm 3$ ( $N = 13$ )	1:3.0 (1:2-1:6)
	1976 (15 days)	$2.4 \pm 0.8$ (0-4)	$7 \pm 2$ ( $N = 9$ )	1:3.2 (1:3-1:5)
	1978 (18 days)	$2.2 \pm 1.0$ (1-5)	$9 \pm 1$ ( $N = 9$ )	1:2.4 (1:2-1:4)

Males of the Idaho ground squirrel guard their mates after copulating, whereas males of Belding's ground squirrels do not. The study areas for both species were about half a hectare.

\* Number of females that were guarded (*S. brunneus*) and mated (*S. beldingi*) on each day of the mating period.

† Nearest neighbour distances between pairs of females that were receptive on the same day.

‡ Mean daily ratio of sexually receptive females to adult males ( $\geq 2$  yr old) during the mating period.

§ Significance levels for Mann-Whitney  $U$  test comparisons between the two species (data from different years combined).

resumed mate-finding and self-advertisement as soon as copulation was complete.

Using an approach based on game theory<sup>34</sup>, I compared the effects of guarding versus searching on the fitnesses of male Idaho and Belding's ground squirrels (Table 2). These analyses suggest that mate guarding in Idaho ground squirrels is evolutionarily stable, largely because (1) locating receptive females is time-consuming for males and requires them to range widely (Table 1), which is dangerous, (2) obtaining sexual access to guarded females is difficult for all but the largest males, (3) unguarded females will mate multiply (Fig. 1a), thus diluting the first male's paternity, and (4) the longest (last) guarding male sires the majority of the litter (Fig. 1b). In contrast, conditions 1, 2, and 4 do not apply in Belding's ground squirrels and, as a result, resumption of mate searching after copulating is the evolutionarily stable state. The phenotypic costs and genetic consequences of mate guarding versus resuming searching merit investigation in other animals. □

**Note added in proof:** D. W. Foltz and P. L. Schwagmeyer have just reported<sup>35</sup> that  $75 \pm 0.8\%$  ( $n = 8$ ) of 13-lined ground squirrel

litters are sired by a female's first mate; this species thus close resembles *S. beldingi* in both male post-copulatory behavior and sperm usage pattern (Table 2).

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**TABLE 2** An analysis of mate guarding behaviour versus resuming mate searching subsequent to copulating for male Idaho ground squirrels and Belding's ground squirrels, using game theory to find the evolutionarily stable state.

<i>S. brunneus</i>	<i>S. beldingi</i>
For guarding to be evolutionarily stable,	For not guarding (resuming searching) to be evolutionarily stable,
(1) $E(G, G) > E(N, G)$	$E(N, N) > E(G, N)$
Is it? If so,	Is it? If so,
(2) $[(g)(\Delta p)(l)]/t_g > [(1 - \Delta p)(l)]/t_s$	$[(1 - \Delta p)(l)]/t_s > [(g)(\Delta p)(l)]/t_g$
(3) $[(0.73)(0.83)(5.20)]/2.90 > [(0.17)(5.20)]/1.30$	$[(0.43)(4.40)]/0.40 > [(1)(0.57)(4.40)]/3.50$
(4) $1.1 > 0.7$	$4.7 > 0.7$
(units: offspring per hour)	(offspring per hour)
<b>Conclusions</b>	
Guarding is evolutionarily stable	Searching is evolutionarily stable

Schwagmeyer and Parker<sup>7</sup> adapted the 'optimal diet' model of MacArthur and Pianka<sup>36</sup> to explore the relative payoffs of mate guarding versus resuming searching by male 13-lined ground squirrels (*S. tridecemlineatus*), a species in which males do not guard. They showed that guarding a female who has just been mated (G) is evolutionarily stable against invasion by non-guarding behaviour (N) when, in a population of guarders, the expected reproductive benefits per unit time from guarding  $E(G, G)$ , exceed the expected gains per unit time from leaving the female and resuming searching  $E(N, G)$ , (inequality 1 for *S. brunneus*). Likewise, a non-guarding population (for example, *S. beldingi*) is resistant to the invasion of guarding behaviour when  $E(N, N) > E(G, N)$ . Expected gains from G and N behaviour depend on the parameters in inequality 2. For *S. brunneus*, these parameters are:  $g$ , the probability that a male can successfully guard his mate from other males, estimated as:  $19/26 = 0.73$  (assuming that overthrown males gain no further matings);  $\Delta p$ , the increase in the probability of paternity due to guarding, calculated as:  $1 - [\text{probability of paternity if the male does not guard}] = 1 - [(0.2) \times (\text{the likelihood that the abandoned female mates once more}) + 0.1 \times (\text{the likelihood that she mates twice more})] = 1 - [(0.2)(5/7) + (0.1)(2/7)] = 0.83$ ; see Fig. 1;  $l$ , the average litter size (5.2);  $t_g$ , the mean length of time a female is guarded (2.9 hours); and  $t_s$ , the mean time it takes a searching male to acquire a subsequent mate (estimated as 1.3 hours, the time it took defeated males to locate a new female). Note that both  $t_g$  and  $t_s$  are estimated conservatively relative to the inequality being examined; furthermore, predation risks, which make searching even more unfavourable, are not considered in the model. For *S. beldingi* the parameters in inequality 2 are defined similarly, but calculated differently:  $g$  is assumed to be 1.0 (that is, if males did guard females, they would do so effectively; this is the most conservative assumption relative to the inequality);  $\Delta p$  is calculated from the relative frequency of mating with 1–5 males<sup>33</sup> and the probability of paternity of the first male given that the female he leaves mates with 0–4 males subsequently (these values are  $[(0.16)(1) + (0.26)(0.6) + (0.26)(0.3) + (0.26)(0.1) + (0.06)(0)] = 0.43$ , so  $\Delta p = 0.57$ ),  $l$  is 4.4 (ref. 31);  $t_g$  is assumed to be the length of the receptive period (3.5 h; ref. 30); and  $t_s$  is 0.4 hours.

## Foraging specialization without relatedness or dominance among co-founding ant queens

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**HYPOTHESES on the evolution of sociality in the Hymenoptera have focused on two non-exclusive selective processes. First, individuals may help relatives to enhance inclusive fitness (k selection<sup>1,2</sup>). Second, group living may be so highly advantageous that competitively inferior individuals are forced into subordinate roles through social competition<sup>3–7</sup>; in this hypothesis, subordinates help dominants in the expectation that they may benefit from the group's resources if the dominants lose status or die. Mai**

social Hymenoptera associate predominantly with close relatives<sup>8,9</sup>, which precludes an effective comparison of kin selection and social competition. Here we report on the existence of foraging specialists among unrelated co-foundresses of the leaf-cutter ant *Acromyrmex versicolor*; such task specialization leaves the forager at a relative fitness disadvantage within her foundress association. Contrary to the predictions of social competition theory, individuals specialize independently of competitive ability (as measured by relative body size) or reproductive status (as measured by ovarian condition) and without conflict. The selective basis of foraging specialization may lie in the intense competition that occurs among newly founded colonies engaged in reciprocal brood raiding.

In many ant species colonies are initiated by multiple foundresses<sup>10</sup>. As in other co-founding ants, *A. versicolor* foundresses do not associate preferentially by collection locale in laboratory experiments, suggesting that associations need not consist of close relatives<sup>11,12</sup>. To confirm this, we estimated within-group relatedness<sup>13,14</sup> from 26 field-caught foundress associations using allozymes as genetic markers. Of 30 tested, one locus was polymorphic<sup>14</sup>. Co-foundresses were no more closely related than randomly selected queens ( $\hat{r} = -0.12$ , s.e. = 0.03, estimated by a jack-knife over groups<sup>15</sup>). The negative  $\hat{r}$  and small s.e. imply that genotypes are distributed more evenly across groups than would be expected under random association. The negative  $\hat{r}$  is probably a sampling artefact caused by small group size ( $N = 3.8$ ) and unequal allele frequencies; we do not infer that kin avoid each other<sup>16</sup>. We conclude that associations constitute random samples of the population, precluding kin selection as an explanation for social traits<sup>17-19</sup>.

Among co-founding ants, *A. versicolor* is unique in that foundresses regularly leave the nest to forage before the emergence of the first workers. Foragers are exposed to greater risks of predation and parasitization, and to greater thermal and physical stresses than their sedentary nest-mates<sup>20-22</sup>. Nonetheless, leaves procured through foraging are shared communally in a single fungus garden. Foraging thus enhances colony fitness at the expense of a forager's relative intracolony fitness<sup>18,19</sup>. As among co-founding wasps, foraging is a task in which specialization might indicate an incipient reproductive division of labour<sup>5,23</sup>, a likely precursor to the evolution of permanent worker sterility which is characteristic of most social hymenoptera<sup>5,20,23,24</sup>.

To study the distribution of foraging effort within colonies, we established eleven colonies, each with three newly mated foundresses collected immediately after a mating flight on 23 September 1987 at North Scottsdale, Arizona. To ensure substantial size variation within each association, the mass of each foundress in an association was arranged to be at least  $\pm 1$  standard deviation (equal to 1.04 mg) from the mass of the other two foundresses. Foundresses were marked, housed in 'ant

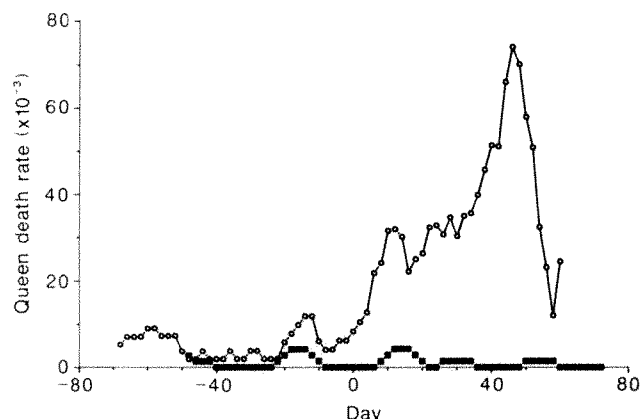


FIG. 1 Comparative rates of foundress mortality in *A. versicolor* (squares) and the ant *Veromessor pergandei* (circles), which displays more typical behaviour for cooperatively founding ants (data for *V. pergandei* from ref. 25). Queen death rate is calculated as the percentage of all the foundresses alive at the start of a 2-day period who died during that period; it is displayed as a 3-point moving average. All observations in each nest are standardized to day of first worker emergence (day 0) in that nest. *V. pergandei* and most other cooperatively founding species reduce to secondary monogyny soon after first worker emergence<sup>28</sup>.

farms' and offered fresh native vegetation in attached foraging arenas, according to standard procedures<sup>25</sup>. Farms and associated arenas were placed on a mechanized circular surface which rotated under a video camera. Foraging trips taken by each co-foundress in each association were censused from video records of 6-hour foraging sessions (57 sessions over 14 weeks). Sessions began immediately after colony formation and ended when workers assumed foraging tasks. Farms were not offered food between sessions. In each association virtually all foraging trips (average was  $165 \pm 69$  trips per farm) were performed by a single foundress ( $P < 0.001$  in each case); the only exceptions occurred in two associations where a single secondary forager appeared after the death of the first foraging specialist.

Another 34 farms were similarly established to see if specialization in foraging was a function of potential competitive ability, as measured by relative size. These farms were spot-sampled 8 times per day for 15 weeks to estimate the number of foraging trips taken by each foundress. Chi-square analyses indicated the existence of a foraging specialist in 44 of the 45 farms in the two samples combined; the role of foraging specialist was independent of initial relative size (Table 1). At the conclusion of these experiments, all co-foundresses from 18 associations were dissected to measure primary oocyte number and size

TABLE 1 Social correlates of reproductive status among *A. versicolor* co-foundresses

	Relative initial mass*			Foraging status	
	Large	Medium	Small	Forager	Non-foragers
Forager	18	16	10		
		$(X^2 = 2.36; NS)$			
Number of primary oocytes	8.2	8.6	8.4	8.6	8.5
( $\pm$ s.d.; N)	(2.9; 18)	(1.9; 18)	(2.1; 18)	(2.2; 18)	(1.9; 35)
		$(F = 0.01; NS)$			$(t = 0.17; NS)$
Primary oocyte length, mm	0.51	0.53	0.56	0.52	0.54
( $\pm$ s.d.; N of queens)	(0.11; 16)	(0.12; 16)	(0.17; 16)	(0.10; 16)	(0.12; 32)
		$(F = 0.70; NS)$			$(t = 0.38; NS)$
Number of total oocytes	18.56	20.28	19.50	20.37	18.94
( $\pm$ s.d.; N of queens)	(13.32; 18)	(16.43; 18)	(14.56; 18)	(15.34; 19)	(14.32; 35)
		$(F = 0.06; NS)$			$(t = 0.34; NS)$

\* Each foundress in each association was  $\geq 1$  s.d. in initial mass from the other 2 foundresses in that association, such that the total number of 'large', 'medium' and 'small' foundresses was the same over all foundress associations.



(routine assays of reproductive conditions<sup>20,26,27</sup>). Reproductive condition was independent of body size and foraging specialization (Table 1); all foundresses had well developed ovaries and therefore presumably functioned as queens. During spot sampling, farms were examined for evidence of aggression that might induce a 'loser' to become a foraging specialist; in addition, the interiors of two colonies were videotaped for an average of 79 h per colony. We never saw aggression among foundresses, nor acts of ritualized dominance such as occur among some Polistine wasps<sup>5,23,27</sup> (for example, asymmetric antennation, allogrooming or trophallaxis). Access to brood or fungus, the principal resources of a colony, was never contested by, nor denied to, any co-foundress. Unlike other cooperatively founding ants<sup>25,28</sup> and some bees<sup>20</sup> and wasps<sup>29</sup>, *A. versicolor* foundresses do not fight on first worker emergence (Fig. 1), and they share resources cooperatively for at least 1.5 yr in laboratory colonies.

The primary benefit of Hymenopteran foundress associations is believed to be defence against enemies<sup>20,27,30</sup>; in some ants, the enemy is conspecific. Newly founded nests are often clumped spatially, and they engage in reciprocal brood raids; colonies with more workers (and queens to produce them) tend to prevail<sup>10,25,31</sup>. This pattern is seen clearly in *A. versicolor*, where newly founded colonies are highly clumped<sup>12</sup>, yet adult colonies are territorial<sup>32</sup>; from a group of new colonies, only a single adult colony will survive. We marked workers in 16 ant farms and paired them to common foraging areas. Brood, leaf and fungus raids occurred. Of 163 raids, 131 involved marked workers; these workers always displayed fidelity to their natal colony. Fighting among workers, and between workers and foreign queens, was common. In each pairing only a single colony survived.

As only one colony from an initial cluster of colonies will survive to adulthood, relatively small differences in colony efficiency could cause large differences in ultimate colony reproductive success. An increase in foraging efficiency should translate to a similar increase in quality of initial raiding force and, ultimately, success in eliminating rivals. Foraging social insects enhance their efficiency through experience<sup>26,33,34</sup>. An *A. versicolor* foraging queen gains experience as she gathers leaves; to refuse further foraging squanders the former resource. Yet each queen should prefer her colony mates to forage, but the simultaneous expression of such preferences would reduce colony fitness; energy and time spent in intragroup conflict over task allocation would limit the colony's future competitive potential<sup>11,35</sup>. Under these circumstances, a foundress may be relatively indifferent as to whether she is the one that suffers the mortality risk associated with foraging, because the colony will either succeed as a unit or (more probably) fail as a unit. □

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## Glycine enhances NMDA-receptor mediated synaptic potentials in neocortical slices

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ONE class of excitatory amino-acid receptors, the *N*-methyl-D-aspartate (NMDA) receptors, mediates transmission at a small, but important, group of synapses in the neocortex<sup>1–3</sup>. These receptors are implicated in neuronal plasticity during development in young mammals and in memory acquisition in adults<sup>4–6</sup>. Recently, responses of isolated membrane patches to NMDA were shown to be greatly enhanced by glycine<sup>7</sup>. This, together with the demonstration that the strychnine-insensitive glycine-binding site is distinct from, but linked to, the NMDA receptor<sup>8</sup> has excited intense interest in glycine as a synaptic modulator. Before proposing a physiological function, however, it is important to determine whether glycine could enhance synaptic responses to NMDA receptor activation in intact, adult tissue. An earlier study<sup>9</sup> failed to demonstrate enhancement of NMDA responses when glycine was applied and it was proposed that in intact tissue the high-affinity glycine site was already saturated by endogenous glycine. It remained possible that glycine concentrations can be maintained at low levels close to synaptic receptors. We have examined responses of neurons in slices of adult neocortex to focal applications of excitatory amino acids and glycine and report enhancement by glycine of NMDA receptor-mediated excitatory postsynaptic potentials.

So that small synaptic responses could be recorded intracellularly under controlled conditions, we used slices of adult neocortex, which does not contain any strychnine-sensitive glycine-binding sites<sup>11</sup>. The perfusing fluid was delivered at a low flow rate (less than 0.3 ml min<sup>-1</sup>) to allow metabolic products, transmitters and modulators to be controlled by natural release, degradation and uptake processes, rather than by the perfusion system.

In all cells tested in which rapid-onset, repeatable responses to electrophoretically applied excitatory amino acids could be evoked, glycine enhanced responses to NMDA (mean increase in amplitude 100.00 ± 65.75% s.d., range 22–284%, *n* = 38), but produced no significant change in responses to quisqualate (mean increase 4 ± 28.54%, range, 50% decrease to 20% increase). The effective glycine concentration is not known, but maximal effects were produced with ejection currents of less than 4 nA (Fig. 1). The responses when glycine was dissolved in acidic and alkaline solutions were similar. The effects of

glycine on NMDA responses were reversed readily when application was terminated. They were rapidly and reversibly blocked by kynurenate, an endogenous antagonist at the high-affinity glycine receptor<sup>9-10</sup>, applied electrophoretically ( $n=29$ ) or in the bathing medium ( $n=3$ ) (50–100  $\mu\text{M}$ , Fig. 1), but were unaffected by strychnine. Effective glycine currents produced no measurable change in resting potential or input resistance.

Stimulation of the white matter evoked excitatory postsynaptic potentials (e.p.s.ps) of between 1 and 5 mV. Glycine applied with a current that augmented responses to NMDA but not to quisqualate augmented the e.p.s.ps evoked in 5 of 24 of these cells (see Fig. 2a–e) and depressed the e.p.s.ps in 7 cells, without affecting the response of the cell to injected current. In a further 5 cells, larger applications of glycine (5 to 40 nA ejection current) caused decreases in both e.p.s.p. and current-pulse response (Fig. 2i, j). Glycine produced no change in the e.p.s.ps in the remaining 7 cells. Effects on e.p.s.ps were readily reversible.

All e.p.s.ps that were insensitive to, or depressed by, glycine

had conventional voltage relations and were insensitive to 2-amino-5-phosphonovaleric acid (AP5) ( $n=10/10$ ); they did not seem to contain a NMDA-receptor mediated component (Fig. 2h, k, l). In contrast, the 5 e.p.s.ps that were augmented by glycine had voltage relations that were typical of neuronal responses to NMDA<sup>1,2</sup> (Fig. 2f) and were depressed by AP5 ( $n=3/5$ ) (Fig. 2a–e). Although glycine produced a relatively small increase in the total e.p.s.p., it produced a substantial increase in the AP5-sensitive, NMDA receptor-mediated, component (Fig. 2g). Although it is possible that AP5 applied electrophoretically did not gain access to all NMDA receptors mediating the e.p.s.p., it is probable that a similar population of receptors was affected by both glycine and AP5, because we used submaximal doses of both drugs (assessed by their actions on NMDA responses) and there was no augmentation by glycine in the presence of AP5.

These results indicate that glycine can selectively enhance both neuronal responses to NMDA and NMDA-receptor-medi-

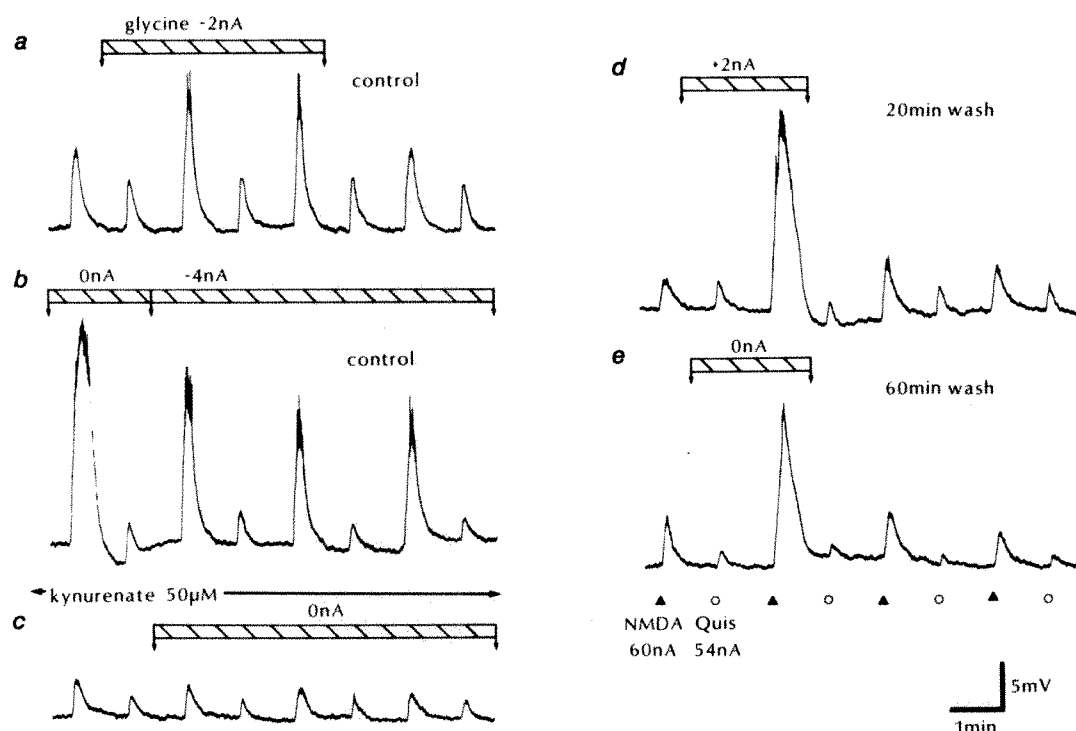


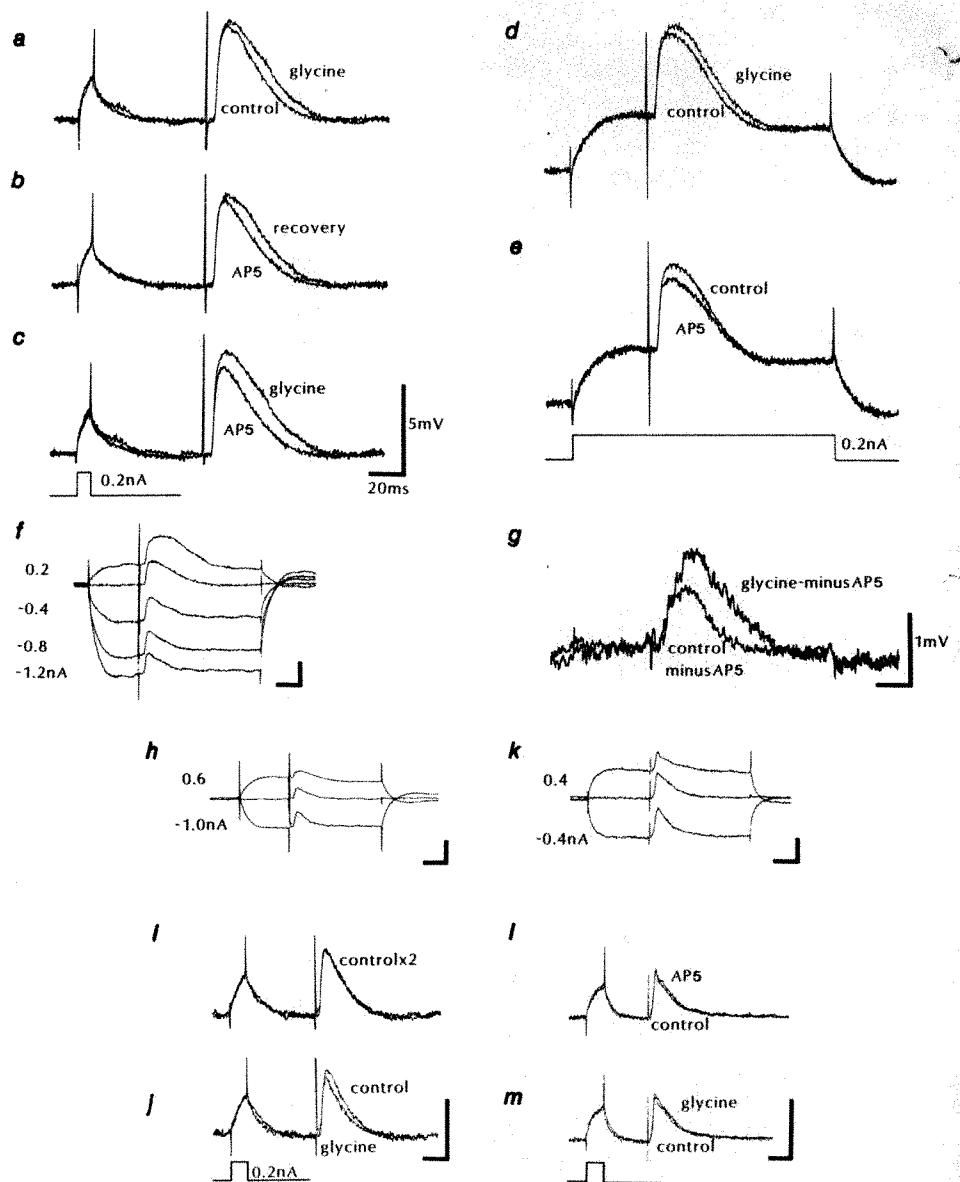
FIG. 1 Augmentation of responses to NMDA by glycine and blocking of the augmentation by kynurenate. Depolarizations of a cortical neuron in response to brief electrophoretic pulses of NMDA (closed triangles) and quisqualate (open circles), recorded intracellularly with conventional current-clamp techniques. The symbols in c and e apply to all records. Amino-acid currents were: Quisqualate, 54 nA, 2s; and NMDA, 60 nA, 2s throughout. In a, electrophoretic application of glycine (hatched bar) with a reduced retaining current (–2 nA) increased the response to NMDA, but not to quisqualate. In b, a larger application of glycine (0 nA retaining current) resulted in a larger augmentation. When the retaining current was increased to –4 nA, responses declined, but remained augmented for three further cycles until the retaining current was returned to its control value (–30 nA) when responses returned, on recovery, to pre-glycine amplitudes (data not shown). After six cycles of enhancement and recovery, kynurenate was applied in the bathing medium (50  $\mu\text{M}$ ). In c, glycine applied 25 min after kynurenate addition, with no retaining current, did not augment NMDA responses. In d, after a 20-min washout, 0 nA glycine was still ineffective (data not shown) but a +2 nA ejection current produced enhancement. In e, after a 60-min washout, 0 nA glycine caused enhancement. All records were obtained at the resting potential –72 mV ( $\pm 2$  mV). Spikes were curtailed by the pen recorder response frequency. A gradual decline in responses to both amino acids occurred

during the 3-h recording period.

**METHODS.** Coronal slices of cingulate/sensorimotor cortex were obtained from adult female Wistar (12) and male Sprague-Dawley (6) rats, as described previously<sup>2</sup>. Intracellular recordings were made from pyramidal neurons in layers II/III. Agonists and antagonists were applied electrophoretically from a 5-barrel pipette whose tip was within 50  $\mu\text{m}$  of the recording site. The barrels contained: NMDA, quisqualate (both 200 mM, pH 8), glycine (200 mM, pH 3 in 30 cells; 50 mM, pH 3 in 3 cells; 50 mM, pH 8 in 5 cells) and kynurenate (50 mM, pH 8) or D-AP5 (5 mM, pH 8) and NaCl (200 mM, balance barrel). Barrel resistances ranged from 70 to 120 M $\Omega$  in pipettes with which significant augmentation was achieved. Excitatory amino acids were applied in brief (1–2s) pulses (5–60 nA), repeated in an electronically controlled cycle with interpulse intervals of between 20 and 90 s. Glycine was applied continuously for 1 to 30 cycles either by reducing the retaining current (to 0–6 nA), or with low ejection currents (0 to 2 nA). Retaining currents for glycine of less than 10 nA resulted in a gradual loss of the augmentation of NMDA by glycine. Kynurenate ejection currents of between 2 and 20 nA rapidly and reversibly blocked the augmentation of NMDA responses by glycine. Larger currents and bath applications, of between 50 and 100  $\mu\text{M}$  reduced responses to both NMDA and quisqualate. In one experiment (three cells) strychnine (5–15  $\mu\text{M}$ ) was bath-applied.

FIG. 2 E.p.s.p.s recorded intracellularly in three cortical neurons (*a* to *g*, *h* to *j* and *k* to *m*). In *a*, the e.p.s.p. is augmented by glycine application (0 nA). After recovery (*b*), the e.p.s.p. is depressed by AP5 (10 nA). This dose of AP5 depressed responses to NMDA by more than 80%, but did not reduce quisqualate responses. The e.p.s.p.s recorded in the presence of glycine and of AP5 are superimposed in *c*; for comparison. In *a*, *b*, *c*, small injected current pulses (0.2 nA), see lower record in *c*, precede stimuli. Note the lack of effect of either AP5 or glycine on the rising phase of the e.p.s.p., which may be due to activation of non-NMDA receptors. We repeated this procedure (*d*, *e*) but with e.p.s.p.s superimposed on longer injected current pulses (0.2 nA, lower record in *e*). These responses were then digitally subtracted and amplified to reveal the AP5-sensitive component under control and glycine conditions (superimposed in *g*). *f*, The unconventional voltage relation of this e.p.s.p., which is typical of responses mediated at least in part by NMDA receptors<sup>1-3,12</sup>. Stimuli were superimposed on injected current pulses (amplitude of pulses given to the left). *h*, *k*, The conventional voltage relations of two other e.p.s.p.s. Three control averages of the e.p.s.p. shown in *h* are illustrated in *i* and in the control record *j*. This e.p.s.p. was depressed by glycine (10 nA ejection current) and there was a concurrent, if smaller, decrease in the voltage response to current injection. The e.p.s.p. shown in *k* was not significantly reduced by AP5 (*l*) nor augmented by glycine (2 nA ejection current; *m*). All records were obtained from resting potential: *a* to *g* -70 mV, *h* to *j* -72 mV, *k* to *m* -79 mV. All scale bars: 5 mV (vertical) and 20 ms (horizontal) except for *g* in which the vertical scale bar is 1 mV. *a*-*e*, *g*, *i*, *j*, *k*, *l*, *m* are averages each of 16 sweeps. *f*, *h* and *k* are averages each of eight sweeps.

**METHODS.** In six experiments, electrical stimulation ( $<1 \mu\text{A}$ ,  $<0.2 \text{ ms}$ , 1 every 5 s) of the underlying white matter was used to evoke small e.p.s.p.s. E.p.s.p.s and voltage responses to small, brief, injected current pulses were averaged (eight to 16 sweeps). Any given response was considered acceptable when three successive averages were similar in shape and amplitude. Augmentation of e.p.s.p.s by glycine, or their depression by AP5 were accepted only when responses recovered to pre-



drug levels and the effect could be repeated. For example, the e.p.s.p. illustrated in *a* to *g* was exposed to three glycine and two AP5 applications and recovered from each. Voltage relations for evoked e.p.s.p.s were obtained by superimposing e.p.s.p.s on longer duration injected current pulses.

ated e.p.s.p.s in adult neocortical slices. As the pyramidal neurons of the cortex receive many conventional, non-NMDA-receptor-mediated inputs, the contribution made by NMDA receptors to their total excitatory input is very small. Even those e.p.s.p.s that are mediated by NMDA receptors usually contain a non-NMDA-receptor-mediated component<sup>2</sup> (see also ref. 12). It is therefore unlikely that changes in local glycine concentration will dramatically change the overall excitatory drive to these cells. But because NMDA receptors are involved in neuronal plasticity, the present demonstration that NMDA-receptor-mediated responses can be enhanced by exogenous glycine indicates that glycine has an important modulatory role. □

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# Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine

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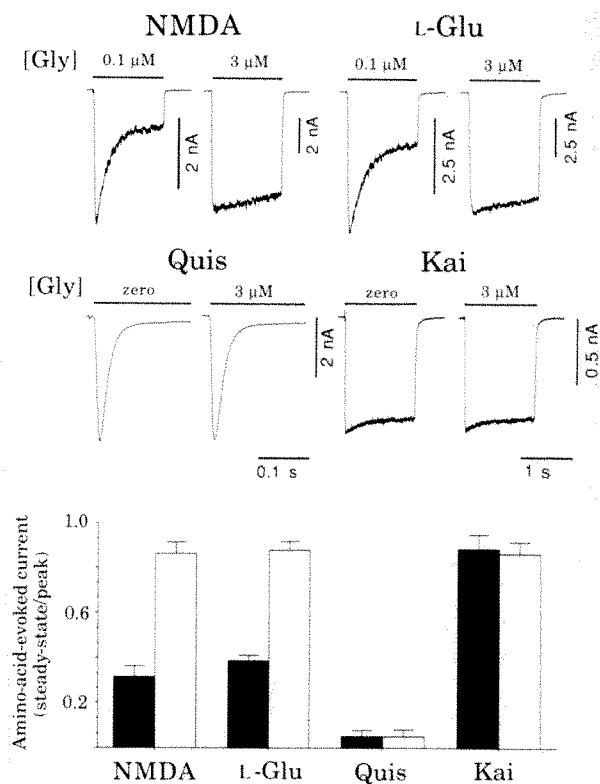
**RESPONSES** to the excitatory amino acid *N*-methyl-D-aspartate (NMDA) are markedly potentiated by nanomolar concentrations of glycine<sup>1-3</sup>. This is due to the action of glycine at a novel strychnine-resistant binding site with an anatomical distribution identical to that for NMDA receptors<sup>4,5</sup>, suggesting that the NMDA receptor channel complex contains at least two classes of amino-acid recognition site. Antagonists at the glycine-binding site associated with NMDA receptors act as potent non-competitive antagonists<sup>6,7</sup>, but do not alter the mean open time or conductance, as estimated by fluctuation analysis<sup>8</sup>. The mechanisms by which glycine acts on NMDA receptors are unknown, but single-channel recording experiments show an increase in opening frequency with no change in mean open time or conductance<sup>7</sup>, suggesting that glycine could regulate transitions to states that are intermediate

between binding of NMDA receptor agonists and ion-channel gating. It has been suggested that glycine acts as a co-agonist at the NMDA receptor, and that responses to NMDA cannot be obtained in the complete absence of glycine<sup>9</sup>, but in these experiments the response to NMDA was measured at equilibrium, and it is unlikely that sufficient temporal resolution was achieved to detect rapid alterations in receptor gating. Using a fast perfusion system we find that glycine regulates desensitization at NMDA receptors; this has a major effect on the response to NMDA measured at equilibrium, as would occur with slower applications of agonist. Reduction of NMDA receptor desensitization by glycine provides an example of a novel mechanism for regulation of ion-channel activity.

We observed that fast applications of excitatory amino acids to hippocampal neurons in dissociated culture produced three patterns of response consistent with activation of kainate, NMDA and quisqualate receptors<sup>10</sup>: responses to kainate showed little or no desensitization; responses to NMDA desensitized relatively slowly with a time constant of around 250 ms; responses to quisqualate desensitized very rapidly, with a time constant of around 10–20 ms or less. In subsequent experiments (Fig. 1) we found that desensitization at NMDA receptors, but not at kainate or quisqualate receptors, was sensitive to changes in the concentration of glycine, such that the desensitization of responses to NMDA was greatly reduced with 3 or 10  $\mu$ M glycine. Similar effects were observed with activation of NMDA receptors by both NMDA and L-glutamate, whereas D-serine and D-alanine (3  $\mu$ M) were able to substitute for glycine in preventing desensitization.

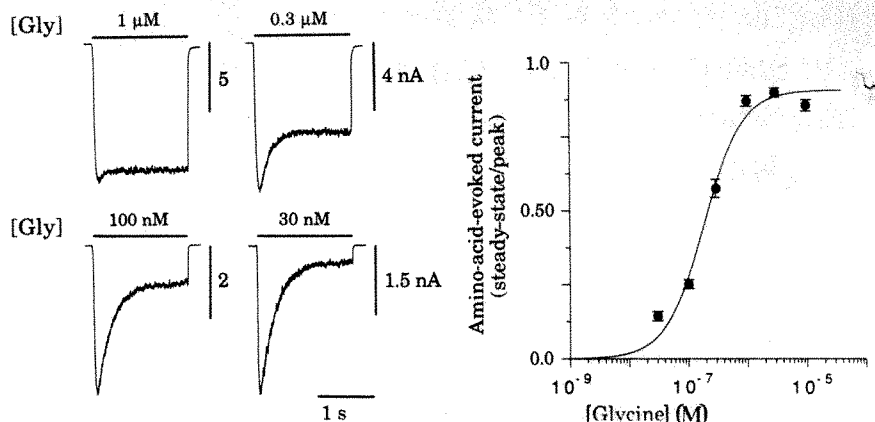
**FIG. 1** Glycine regulates desensitization at NMDA receptors, but not at kainate (Kai) or quisqualate (Quis) receptors. Responses were evoked by fast applications of NMDA (100  $\mu$ M), L-glutamate (10  $\mu$ M), quisqualate (40  $\mu$ M) and kainate (50  $\mu$ M) with 0.1  $\mu$ M and 3  $\mu$ M glycine (NMDA and L-glutamate) or zero and 3  $\mu$ M glycine (quisqualate and kainate) using whole-cell recording under discontinuous voltage clamp. The responses for NMDA and L-glutamate recorded with 0.1  $\mu$ M glycine are plotted at twice the gain of the responses recorded with 3  $\mu$ M glycine, to emphasize the effect of glycine on desensitization. The time base for responses evoked by quisqualate is 10 times faster than for the other agonists. The onset of desensitization of the responses to NMDA and L-glutamate recorded with 0.1  $\mu$ M glycine could be fitted to exponential functions of time constant 220 and 305 ms respectively. The onset of desensitization of the response to quisqualate did not change with zero or 3  $\mu$ M glycine, and could be fitted to an exponential function of time constant 17.6 ms. The responses to NMDA and L-glutamate were recorded in the same neuron, and emphasize the difference in potency of L-glutamate and NMDA, which was confirmed in separate experiments (not shown) in which complete concentration response curves were obtained for each agonist; at these doses the responses to NMDA and L-glutamate are near maximum. The residual desensitization of responses to NMDA recorded with 3  $\mu$ M glycine was fairly variable and essentially absent in some experiments; as this process was accelerated on raising the extracellular calcium concentration, or on substituting EGTA for BAPTA as an intracellular calcium buffer, we suggest that it represents a separate process, triggered by a rise in intracellular calcium ion concentration as a result of calcium influx through NMDA receptor channels. The bar graph summarizes results from experiments on nine neurons, and is plotted as steady state (measured at 1.5 s)/peak current; error bars show 1 standard deviation. Responses recorded with 0.1  $\mu$ M glycine are represented by filled columns; responses with 3  $\mu$ M glycine by open columns.

**METHODS.** Experiments used primary cultures of hippocampal neurons from 16–17-day-old mouse embryos plated on a primary culture of glial cells also from mouse hippocampus<sup>15</sup>. Neurons were kept in culture for up to three weeks, but most experiments were performed 4–14 days after plating, as development of the dendritic field made rapid solution changes more difficult to attain in older cells. In experiments in which cells were bathed in 50  $\mu$ M kainic acid and the extracellular sodium concentration stepped from 5 to 165 mM, an inward relaxation developed with a time constant of 7–10 ms indicating that there was rapid perfusion under our experimental conditions. The perfusion apparatus was similar to that used by Johnson and Ascher<sup>14,12</sup> and is described in more detail elsewhere<sup>10</sup>. The extracellular solution contained (in mM): 165 NaCl, 2.5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 glucose, 10 HEPES, pH 7.3, with 400 nM TTX (tetrodotoxin) and 5  $\mu$ M bicuculline methiodide,



and glycine added as indicated. In all experiments, neurons were pre-equilibrated with the required concentration of glycine for 10–20 s before NMDA was applied together with glycine. NMDA and L-glutamate were applied using a modified extracellular solution containing 0.2 mM CaCl<sub>2</sub> to reduce calcium-mediated inactivation of NMDA receptor activity<sup>16,17</sup> and no added Mg<sup>17,18</sup> to prevent channel block. The intracellular solution contained (in mM): 125 CsMeSO<sub>3</sub>, 15 CsCl, 5 CsBAPTA (1,2-bis(o-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid), 0.5 CaCl<sub>2</sub>, 5 MgCl<sub>2</sub> and 2 ATP. Experiments were performed at room temperature (25–27 °C).

**FIG. 2** Concentration-dependent regulation by glycine of NMDA receptor desensitization. Traces show the responses to rapid applications of 100  $\mu$ M NMDA at the glycine concentrations indicated and are plotted so that the peak amplitude of the traces is similar. Desensitization is pronounced at low concentrations of glycine, but essentially absent at high concentrations of glycine. Note the similar time course of the onset of desensitization at different concentrations of glycine. Responses to 30 nM and 100 nM glycine were recorded from the same neuron, responses to 0.3  $\mu$ M and 1  $\mu$ M glycine were recorded from a second neuron. The dose-response curve summarizes results from 13 neurons each of which was tested with four concentrations of glycine. The y axis is a measure of desensitization and shows values calculated as steady-state current (measured at 1.5 s) divided by the peak-current response to NMDA. The data points are fitted by nonlinear regression to the Hill equation (maximum=0.9;  $K_d$ =185 nM;  $n$ =1.3; error bars show 1 s.d. Further analysis of these experiments showed potentiation by glycine of both the peak and steady-state response to NMDA, although because of the effect of glycine on desensitization the potentiation of the steady-state response to NMDA was much larger; on average, the peak

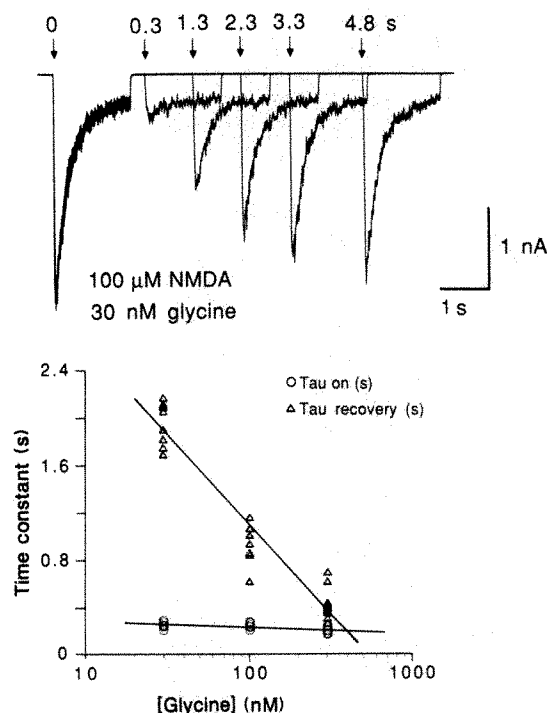


current increased by 3.53 on raising the glycine concentration from 30 nM to 10  $\mu$ M glycine, whereas the steady state current increased by 26.6. One interpretation of this result would be an agonist-evoked transition of the NMDA receptor to a state of lower affinity for glycine, such that in the presence of NMDA or L-glutamate, higher concentrations of glycine are required to maintain ion-channel activity.

Johnson and Ascher have described quantitative experiments on NMDA receptor modulation by glycine in mammalian central nervous system neurons, which suggest that glycine binds to a single site with an affinity constant of about 300 nM<sup>11</sup>; similar estimates have been obtained using binding of [<sup>3</sup>H]glycine to NMDA receptors in membrane preparations<sup>8</sup> or the application of NMDA receptor agonists to mRNA-injected oocytes under voltage clamp<sup>2,3,9</sup>. In our experiments, the potentiation by glycine of NMDA receptor responses measured at steady state (at the end of 1.5-s applications of NMDA) could be described by similar parameters: a 24-fold maximum potentiation of responses to NMDA with 10  $\mu$ M glycine compared with responses measured with 3 nM glycine, with a dissociation constant of 360 nM and  $n$ =1.3, estimated using nonlinear regression to fit the Hill equation to data recorded from 13 neurons (data not shown).

To determine whether glycine regulation of NMDA receptor desensitization occurs over a similar concentration range, we examined responses to rapid applications of NMDA in neurons pre-equilibrated for 10–20 s with 3 nM–10  $\mu$ M glycine (Fig. 2). We used this procedure to allow pre-equilibration of glycine binding, as at sub-micromolar concentrations, diffusion-limited binding of glycine would be expected to markedly slow the interaction of glycine with NMDA receptors if a rapid change in the concentration of both glycine and NMDA was made simultaneously. Our results indicate that the glycine-binding site regulating desensitization of NMDA receptors is titrated over a similar concentration range to that regulating potentiation of responses to NMDA measured at equilibrium, as the dose-response analysis of the degree of desensitization of NMDA receptor responses could be described by a binding model with a dissociation constant of 185 nM,  $n$ =1.3. The requirement that applications of agonist are fast to allow recording of desensitization at NMDA receptors is revealed by inspection of responses recorded at low glycine concentrations ( $\leq$ 100 nM) as, with 100  $\mu$ M NMDA, equilibrium desensitization (measured as the ratio of steady-state/peak current) is more than 75%, and its onset is rapid, being 90% complete within 500 ms.

Our results are in reasonable agreement with the original experiments of Johnson and Ascher<sup>1</sup> as, at the single-channel level, a reduction of desensitization by glycine would probably be recorded as an increase in frequency of ion-channel opening evoked by NMDA receptor agonists if the mean lifetime of desensitized states is long relative to the open and closed time distributions measured during normal gating behaviour of the NMDA receptor, as for nicotinic receptors<sup>12</sup>. The analysis of



**FIG. 3** Glycine increases the rate of recovery from desensitization at NMDA receptors. The traces show a family of currents evoked by paired pulse applications of 100  $\mu$ M NMDA with 30 nM glycine, recorded while the interval between the first and second pulse of NMDA was increased as indicated, to measure the speed of recovery from desensitization. In all cells studied with 30 nM glycine, complete recovery from desensitization required more than five seconds. When the peak amplitude of the response to the second application of NMDA, normalized with respect to the peak amplitude of the first response to NMDA, was plotted as a function of time, recovery from desensitization could be described by a single exponential function (tau recovery) which became faster as the concentration of glycine was increased; in the example shown, the time constant of recovery from desensitization was 3.1 s. The onset of desensitization could also be fitted by a single exponential function (tau on) but the time constant was much less sensitive to the concentration of glycine; in the traces shown, desensitization developed with a time constant of 277 ms. A graph showing the results of exponential fits to the onset and recovery from NMDA receptor desensitization in 25 experiments similar to that illustrated above is shown for 30, 100 and 300 nM glycine, and clearly shows the effect of glycine on the rate of recovery from desensitization, with minimal change in the time of onset of desensitization.

whole-cell currents provides one way to examine this possibility; we used paired pulse applications of NMDA at several concentrations of glycine to measure the rates of onset and recovery from desensitization as a function of glycine concentration (Fig. 3). Over the range 30 to 300 nM glycine, there is little change in the rate of onset of desensitization, but a dramatic speeding up of the rate of recovery from desensitization.

Although previous experiments have used fast applications of NMDA to study modulation by glycine, changes in desensitization were not reported<sup>2</sup>; this probably reflects application of agonist from a point source to a large area of membrane (*Xenopus laevis* oocytes used for voltage-clamp experiments are typically  $\geq 1$  mm in diameter), making it very difficult to achieve a rapid and uniform application of agonist, thus masking desensitization at NMDA receptors. Second, we have found that calcium influx through NMDA receptors triggers secondary mechanisms, which leads to glycine-resistant desensitization; to avoid this, we used a low extracellular concentration of calcium in all our experiments.

Further analysis of the mechanism of action of glycine on NMDA receptor channels will ultimately require a much deeper understanding of the receptor-gating process than has been achieved for any agonist-activated channel. Nonetheless, our results indicate that a major component of the action of glycine on NMDA receptors involves speeding up the rate constant of recovery from desensitization, such that NMDA receptor channels can open more frequently at equilibrium because the rate of escape from a long-lived desensitized state increases as the concentration of glycine is raised. To our knowledge, these are the first results describing regulation of ion-channel activity by an allosteric mechanism involving reduction of desensitization. Indeed, an initial inspection of the common models for desensitization at the much better characterized nicotinic acetylcholine receptor<sup>3</sup> suggests that processes that increase the degree of occupation of the agonist recognition site or the frequency of ion-channel activation would be expected to increase desensitization, as binding of agonist, receptor activation and desensitization are all tightly coupled processes. Experiments on GABA<sub>A</sub> (gamma-aminobutyric acid) receptor channels have shown that the benzodiazepine modulator chlordiazepoxide, which enhances responses to GABA, also increases the degree of desensitization at any given dose of GABA<sup>14</sup>. In contrast, modulation of desensitization at nicotinic acetylcholine receptors by substance P occurs without any change in the amplitude of the initial response to agonist<sup>20</sup> and differs from the effect of glycine described here in that it occurs by acceleration of the onset of desensitization, with no change in the rate of recovery from desensitization. As a result, substance P markedly inhibits responses to acetylcholine measured at equilibrium. Together, the effects of glycine and substance P provide dramatic examples of how modulation of desensitization can regulate the response to neurotransmitters.

Whether desensitization at NMDA receptors occurs under physiological conditions will be determined by the time course of the excitatory synaptic transmitter concentration transient at synaptic boutons. The decay time constant of the NMDA receptor component of synaptic current is unusually long and can last for hundreds of milliseconds<sup>21</sup>, which is within the period over which desensitization occurs in biophysical experiments of the type described here. The long time course for the synaptic current probably reflects the continued presence of transmitter, as we have found rapid deactivation of NMDA receptor current (time constant  $\leq 10$  ms) on removal of agonist in concentration jump experiments. This suggests that the regulation by glycine of NMDA receptor desensitization *in vivo* could have important functional consequences. □

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## Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells

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THE molecular mechanisms by which  $\text{Ca}^{2+}$  controls a variety of ion transport-associated cellular functions in higher plant cells<sup>1</sup>, including movements of stomatal pores, remain unknown. Stomatal pores regulate the gas exchange in leaves. Openings of stomatal pores are mediated by an increase in the intracellular potassium and anion content of guard cells<sup>2,3</sup>. Voltage-dependent  $\text{K}^+$  channels<sup>4–7</sup> and hyperpolarizing  $\text{H}^+$  pumps<sup>8,9</sup> have been identified as mechanisms controlling stomatal movements. But depolarizing mechanisms required for  $\text{K}^+$  efflux during stomatal closing remain unknown. Indirect evidence suggests that  $\text{Ca}^{2+}$  triggers stomatal closing and inhibits stomatal opening<sup>10–12</sup>. Using patch-clamp techniques<sup>13</sup>, we show here that elevated (micromolar) concentrations of cytosolic  $\text{Ca}^{2+}$  in guard cells block inward rectifying  $\text{K}^+$  channels. Furthermore, elevation of cytosolic  $\text{Ca}^{2+}$  leads to the activation of a voltage-dependent depolarizing conductance with a permeability to anions. The  $\text{Ca}^{2+}$ -induced modulation of ion channels reported here could provide a molecular basis for  $\text{Ca}^{2+}$ -dependent regulation of stomatal movements in leaves.

Figure 1a illustrates typical voltage-dependent inward and outward conducting  $\text{K}^+$  channel currents<sup>5,6</sup> recorded across the plasma membrane (plasmalemma) of a guard cell using the whole-cell patch clamp technique<sup>13</sup>. When the cytoplasmic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) was buffered to low values of 0.01  $\mu\text{M}$  and 0.1  $\mu\text{M}$ , the inward conducting  $\text{K}^+$  current ( $I_{\text{K}^+, \text{in}}$ ) was activated at potentials more negative than  $-87$  mV ( $\pm 12$  mV,  $\pm$  s.e.m.  $n = 12$ ) (Fig. 1a and c).

Raising the cytosolic  $\text{Ca}^{2+}$  concentration to 1.5  $\mu\text{M}$  resulted in several dramatic changes in the guard cell membrane currents (Fig. 1b). The inward rectifying  $\text{K}^+$  current was inhibited in the normal activation range when  $[\text{Ca}^{2+}]_{\text{cyt}}$  was elevated (Fig. 1b, c). With 1.5  $\mu\text{M}$   $[\text{Ca}^{2+}]_{\text{cyt}}$ , time-dependent inward rectifying currents were activated at potentials more negative than  $-192$  mV ( $\pm 13$  mV,  $\pm$  s.e.m.,  $n = 19$ ) (Fig. 1b). When  $[\text{Ca}^{2+}]_{\text{cyt}}$  was buffered to 9  $\mu\text{M}$ , inward current activation occurred at potentials more negative than  $-196$  mV ( $\pm 13$  mV,  $n = 11$ ). Tail currents<sup>5</sup> of these hyperpolarization-induced currents at elevated  $[\text{Ca}^{2+}]_{\text{cyt}}$  reversed at the  $\text{K}^+$  equilibrium potential, indicating that they

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were carried by  $K^+$  ions (data not shown). Thus, elevation of cytosolic  $Ca^{2+}$  seems to shift the activation potential of  $I_{K^+,in}$  to large negative potentials.

To test this suggestion, single-channel currents were recorded in cell-free outside-out membrane patches<sup>13</sup> (Fig. 2). With low free  $Ca^{2+}$  concentrations ( $0.01 \mu M$ ) on the cytoplasmic side of the membrane, increasingly negative potentials enhanced the probability of channel opening (Fig. 2a). But when  $[Ca^{2+}]_{cyt}$  was elevated, only few discrete single-channel current steps were observed at large negative potentials (Fig. 2b).

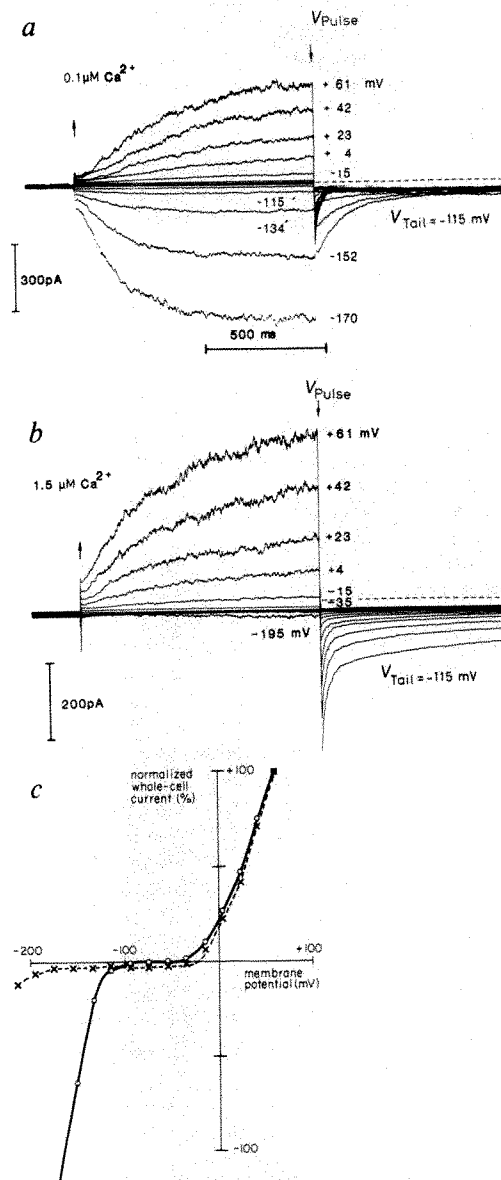
Single-channel conductances recorded at low cytoplasmic  $Ca^{2+}$  ranged from 5 to 8 pS ( $10^{-12} \Omega^{-1}$ ,  $n = 3$ ). Channel conductances recorded at elevated  $[Ca^{2+}]_{cyt}$  were 4–7 pS ( $n = 3$ ). Extrapolation of single-channel current amplitudes with low and elevated  $[Ca^{2+}]_{cyt}$  resulted in reversal potentials close to the  $K^+$ -equilibrium potential ( $E_{K^+} = -54$  mV). These results indicate that the  $Ca^{2+}$ -dependent reduction of  $I_{K^+,in}$  was mediated by a shift in the activation potential of  $I_{K^+,in}$  channels to large negative potentials. Moreover, experiments with excised membrane patches demonstrate that cytoplasmic  $Ca^{2+}$  modulates  $I_{K^+,in}$  channels by directly effecting plasma membrane-bound processes, rather than by interacting with other intracellular factors.

FIG. 1 Effect of cytosolic  $Ca^{2+}$  on guard cell plasma membrane currents in whole-cell recordings. **a**, Inward conducting  $K^+$  currents<sup>4–6</sup> (downward deflections) and outward conducting  $K^+$  currents<sup>5–7</sup> (upward deflections) recorded with  $0.1 \mu M$  free cytosolic  $Ca^{2+}$ . Consecutive one-second voltage steps were applied from a holding potential of  $-75$  mV to pulse potentials ( $V_{Pulse}$ , between the arrows) ranging from  $+61$  mV to  $-170$  mV, as indicated to the right of currents. After  $V_{Pulse}$ , the membrane was stepped to  $-115$  mV ( $V_{Tail}$ ). (The group of rapidly deactivating currents at  $V_{Tail} = -115$  mV corresponded to deactivation of  $I_{K^+,out}$ , whereas the group of slowly deactivating currents corresponded to deactivation of  $I_{K^+,in}$ .) Whole-cell capacitance was  $5.35$  pF. Membrane potential was  $-52$  mV (zero current). **b**, Whole-cell currents measured as described in **a**, with  $1.5 \mu M$  free  $Ca^{2+}$  in the cytoplasm. The membrane potential was  $-20$  mV (zero current). The zero-current level is indicated by the broken line on the right in **(a)** and **(b)**. Whole-cell capacitance was  $5.57$  pF. **c**, Normalized whole-cell currents from cells shown in **(a)** and **(b)** plotted as a function of  $V_{Pulse}$  (open circles:  $[Ca^{2+}]_{cyt} = 0.1 \mu M$ ; crosses:  $[Ca^{2+}]_{cyt} = 1.5 \mu M$ ).

**METHODS.** Guard cell protoplasts were isolated from the lower leaf epidermis of *Vicia faba* as previously described<sup>6</sup> and were bathed during recordings in external solution containing:  $10$  mM potassium glutamate,  $2$  mM  $MgCl_2$ ,  $1$  mM  $CaCl_2$ ,  $1$  mM KOH,  $10$  mM MES 2-[*N*-morpholino]ethanesulphonic acid, pH 5.5, osmolality adjusted to  $480$  mmol  $kg^{-1}$  with D-mannitol. The internal solution, which equilibrated with the cytoplasm, contained:  $100$  mM potassium glutamate,  $2$  mM  $MgCl_2$ ,  $4$  mM KOH,  $10$  mM HEPES,  $4$  mM MgATP, pH 7.2, and D-mannitol, osmolality  $530$  mmol  $kg^{-1}$ . Concentrations of free  $Ca^{2+}$  were buffered in the internal solution by addition of  $0.45$  mM  $CaCl_2$  and  $1.1$  mM  $K_2$ -EGTA in **(a)** and by addition of  $1$  mM  $CaCl_2$  and  $2$  mM 5,5'-dibromobAPTA, tetrapotassium ( $K_4$ -D-Br-BAPTA) (Molecular Probes, Oregon) in **(b)**.  $Ca^{2+}$ -EGTA buffers (dissociation constant  $K_D = 0.147 \mu M$ ) were prepared to compensate for impurities of EGTA<sup>30</sup>.  $Ca^{2+}$  buffers using  $K_4$ -D-Br-BAPTA ( $K_D \sim 1.5 \mu M$ )<sup>30</sup> were corrected for impurities and effects of ionic strength by calibration of internal solutions using  $Ca^{2+}$ -selective electrodes. Voltage-clamp and zero current-clamp recordings were performed with an EPC-7 patch-clamp amplifier (List Electronic) and analysed<sup>6</sup> on a PDP 11/23 computer. The membrane was voltage-clamped to  $-75$  mV for 12 s between consecutive voltage pulses. Liquid junction and series resistance potentials were accounted for<sup>6</sup>. All whole-cell recordings had effective access resistances<sup>5</sup> of  $\leq 10$  M $\Omega$  to ensure efficient control of  $[Ca^{2+}]_{cyt}$ . The temperature was  $22^\circ C$ .

Elevation of  $[Ca^{2+}]_{cyt}$  produced further changes in the ionic currents of guard cells. Depolarizing voltage steps with  $1.5 \mu M$   $[Ca^{2+}]_{cyt}$  enhanced voltage-dependent instantaneous currents (Fig. 1b) in addition to the time-dependent  $K^+$  currents observed at low  $[Ca^{2+}]_{cyt}$ , and repolarization of the membrane to  $-115$  mV ( $V_{Tail}$ ) evoked slow relaxation currents in addition to the rapidly deactivating  $I_{K^+,out}$  tail currents (compare  $V_{Tail} = -115$  mV in Fig. 1a, b). In current-clamp recordings of whole cells dialysed with low  $[Ca^{2+}]_{cyt}$  ( $\leq 0.1 \mu M$ ), the membrane potentials ( $V_m$ ) were never more positive than the activation potential of  $I_{K^+,out}$  ( $V_m = -39 \pm 15$  mV,  $\pm$ s.e.m.,  $n = 11$ ). Interestingly, elevation of cytosolic  $Ca^{2+}$  levels ( $1.5 \mu M$  and  $9 \mu M$ ) often resulted in membrane potentials that were more positive than the activation potential of whole-cell currents (Fig. 1b, c).

To examine the ion selectivity of the  $[Ca^{2+}]_{cyt}$ -dependent depolarizing currents, solutions were prepared that would shift anion equilibrium potentials to more positive values and cation equilibrium potentials to more negative values (see Fig. 3 legend). Furthermore, to abolish  $K^+$  channel currents, we replaced  $K^+$  in the cytoplasmic and bath solutions by minutely permeant  $Cs^+$  ions<sup>6</sup>. With  $0.01 \mu M$   $[Ca^{2+}]_{cyt}$  in whole cells, only small currents were recorded (Fig. 3a), whereas  $Ca^{2+}$ -activated currents appeared with  $1.5 \mu M$   $[Ca^{2+}]_{cyt}$  (Fig. 3b).



Using these  $\text{Cs}^+$  solutions, the membrane potentials ( $V_m$ ) of the cells with elevated  $[\text{Ca}^{2+}]_{\text{cyt}}$  ( $1.5 \mu\text{M}$ ) depolarized to  $+15 \text{ mV}$  ( $\pm 12 \text{ mV}$ ,  $\pm \text{s.e.m.}$ ,  $n = 7$ ). When  $\text{Cs}^+$  ions were replaced by  $\text{K}^+$  ions in the solutions used in Fig. 3, membrane potentials also depolarized with  $1.5 \mu\text{M}$   $[\text{Ca}^{2+}]_{\text{cyt}}$  ( $V_m = +9 \pm 8 \text{ mV}$ ,  $\pm \text{s.e.m.}$ ,  $n = 9$ ). But membrane potentials were substantially more negative when cytosolic  $\text{Ca}^{2+}$  was buffered to low concentrations of  $0.01$  and  $0.1 \mu\text{M}$  ( $V_m = -40.5 \pm 15 \text{ mV}$ ,  $\pm \text{s.e.m.}$ ,  $n = 15$ ).

Although  $[\text{Ca}^{2+}]_{\text{cyt}}$  may also modulate outward  $\text{K}^+$  currents, the  $\text{Ca}^{2+}$ -activated depolarizing currents can be attributed only to  $\text{Ca}^{2+}$  or anion permeation, as the equilibrium potentials of all other ions present were  $0 \text{ mV}$ , or more negative (Fig. 3 legend). Lowering external  $\text{Ca}^{2+}$  from  $1 \text{ mM}$  to  $0.1 \text{ mM}$  did not hyperpolarize the membrane or reduce currents, as seen in Fig. 3b. These results indicate that the  $\text{Ca}^{2+}$ -activated depolarizing currents were not carried by  $\text{Ca}^{2+}$  ions and suggest that membrane depolarizations were induced by a  $\text{Ca}^{2+}$ -activated anion permeability. This suggestion was supported by the finding that the membrane depolarized to between  $+30 \text{ mV}$  and  $+50 \text{ mV}$  when the cytoplasm was loaded with solutions containing  $100 \text{ mM}$   $\text{CsCl}$  and cells were bathed in  $10 \text{ mM}$   $\text{CsCl}$  ( $n = 4$ ).

The  $\text{Ca}^{2+}$ -activated currents and the  $\text{Ca}^{2+}$ -induced depolarizations usually diminished within 2–10 min of establishing whole-cell recordings ('wash-out'). The 'wash-out' of the  $\text{Ca}^{2+}$ -dependent depolarizing currents indicated that other factors may be important for the activation of this conductance.

Our results directly demonstrate that variation of cytosolic  $\text{Ca}^{2+}$  in the range reported to be physiological ( $0.1$  to  $10 \mu\text{M}$ )<sup>14–19</sup>, strongly modulates ion channels in the plasma membrane of a higher plant cell. Extracellular manipulations of  $\text{Ca}^{2+}$  in stomata during physiological stimulation by light and abscisic acid<sup>10–12</sup> have recently indicated that  $\text{Ca}^{2+}$  may exert two separate actions on stomatal movements: first,  $\text{Ca}^{2+}$  is required to initiate stomatal closing<sup>10–12</sup> which relies on reduction of the  $\text{K}^+$  and anion content of guard cells<sup>2,20</sup>; and second,  $\text{Ca}^{2+}$  inhibits stomatal opening<sup>10–12</sup> and the required  $\text{K}^+$  influx<sup>11</sup>.

Previous investigations have provided biophysical and pharmacological evidence that  $I_{\text{K}^+, \text{in}}$  channels represent a major pathway for  $\text{K}^+$  influx during stomatal opening<sup>4–6</sup>. Thus, the inhibition of  $I_{\text{K}^+, \text{in}}$  channels at elevated  $[\text{Ca}^{2+}]_{\text{cyt}}$  reported here may provide a mechanism for the observed prevention of stomatal opening by  $\text{Ca}^{2+}$ . In animal cells, elevation of  $[\text{Ca}^{2+}]_{\text{cyt}}$  can activate outward conducting  $\text{K}^+$  channels<sup>21,22</sup>. Interestingly, both the  $[\text{Ca}^{2+}]_{\text{cyt}}$ -dependent inhibition of  $I_{\text{K}^+, \text{in}}$  found here, and  $\text{Ca}^{2+}$  stimulation of  $\text{K}^+$  channels in animal cells are mediated by hyperpolarizing shifts in the activation potential at increased  $[\text{Ca}^{2+}]_{\text{ext}}$  (Figs 1 and 2; ref. 22).

In addition to reducing  $I_{\text{K}^+, \text{in}}$ , elevation of  $[\text{Ca}^{2+}]_{\text{cyt}}$  led to activation of a voltage-dependent depolarizing conductance

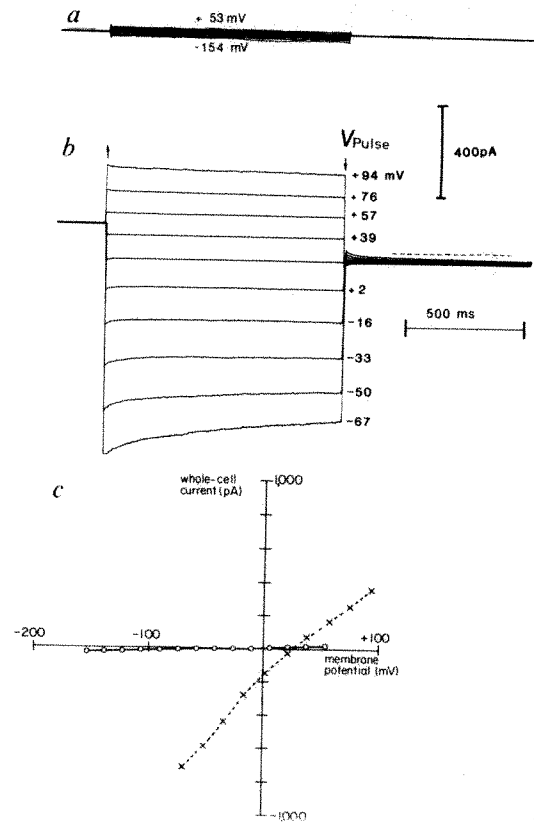
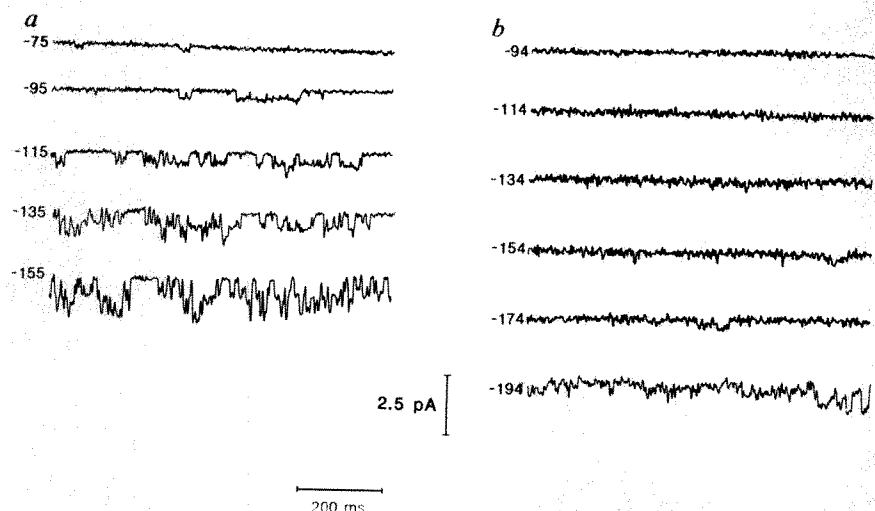


FIG. 3 Effect of cytosolic  $\text{Ca}^{2+}$  on whole-cell currents recorded with  $\text{Cs}^+$  solutions. **a**, Whole-cell currents in response to voltage steps in the range from  $+53 \text{ mV}$  to  $-154 \text{ mV}$  with the cytoplasm exposed to  $0.01 \mu\text{M}$   $\text{Ca}^{2+}$ . **b**, Whole-cell currents in response to voltage steps from  $+48 \text{ mV}$  with  $1.5 \mu\text{M}$  free  $\text{Ca}^{2+}$  in the pipette solution. The zero-current level is indicated by the broken line on the right. In 84% of the guard cell protoplast preparations ( $n = 25$ )  $\text{Ca}^{2+}$ -activated currents as shown in Figs 1b and 3b were detected ('wash-out'). **c**, Current-voltage relationships of cells shown in (a) and (b) (open circles:  $[\text{Ca}^{2+}]_{\text{cyt}} = 0.01 \mu\text{M}$ ; crosses:  $[\text{Ca}^{2+}]_{\text{cyt}} = 1.5 \mu\text{M}$ ). The internal  $\text{Cs}^+$  solution contained:  $80 \text{ mM}$   $\text{Cs}_2\text{-EGTA}$  in (a) and  $1 \text{ mM}$   $\text{CaCl}_2$ ,  $2 \text{ mM}$   $\text{K}_4\text{-D-Br-BAPTA}$  in (b). The external  $\text{Cs}^+$  solution contained:  $8 \text{ mM}$   $\text{Cs-glutamate}$ ,  $0.5 \text{ mM}$   $\text{CsCl}$ ,  $1 \text{ mM}$   $\text{CaCl}_2$ ,  $10 \text{ mM}$   $\text{HEPES}$ ,  $\text{pH } 7.2$ , with osmolalities as in Fig. 1. Using these solutions, the  $\text{Cl}^-$  equilibrium potential ( $E_{\text{Cl}^-}$ ), after correction for ionic activities<sup>6</sup>, was shifted from  $0 \text{ mV}$ , as was the case for solutions used in Fig. 1b, to  $+54 \text{ mV}$  in Fig. 3b ( $E_{\text{glutamate}} = +54 \text{ mV}$ ;  $E_{\text{Ca}^{2+}} > +82 \text{ mV}$ ), whereas  $E_{\text{H}^+}$  was shifted from  $+100 \text{ mV}$  to  $0 \text{ mV}$  and the equilibrium potential for free divalent cations was shifted from  $+5 \text{ mV}$  to  $-9 \text{ mV}$  (compare solutions in Figs 1 and 3).

FIG. 2 Cytosolic  $\text{Ca}^{2+}$  modulates single  $I_{\text{K}^+, \text{in}}$  channels in outside-out plasma membrane patches. The membrane potential of outside-out patches was continuously polarized to negative values at which the time-dependent activation of single  $I_{\text{K}^+, \text{in}}$  channels has previously been described<sup>5</sup>. **a**, Recordings of single  $\text{K}^+$  channel currents at  $0.01 \mu\text{M}$   $[\text{Ca}^{2+}]_{\text{cyt}}$ . Increasingly negative holding potentials, as indicated to the left of the current traces, led to increased opening of  $I_{\text{K}^+, \text{in}}$  channels. Small patch pipette tips were used to reduce the number of channels in the patch (tip resistance  $10 \text{ M}\Omega$  in  $150 \text{ mM}$   $\text{KCl}$  solutions<sup>4</sup>). **b**, Current recordings in an outside-out membrane patch with  $9 \mu\text{M}$  free  $\text{Ca}^{2+}$  on the cytoplasmic membrane side. Cytosolic  $\text{Ca}^{2+}$  was buffered to  $9 \mu\text{M}$  by addition of  $0.66 \text{ mM}$   $\text{CaCl}_2$  and  $0.77 \text{ mM}$   $\text{K}_4\text{-D-Br-BAPTA}$ . Solutions are described in the legend to Fig. 1.



with a permeability to anions. As mature guard cells are uncoupled from neighbouring epidermal cells<sup>23</sup>, the observed  $[Ca^{2+}]_{\text{cyt}}$ -induced depolarizations to about +10 mV may take place in guard cells embedded in their natural environment. Such depolarizations have been estimated to be sufficient to drive physiological rates of  $K^+$  efflux through  $I_{K^+, \text{out}}$  channels<sup>4,5</sup>.  $Ca^{2+}$  can therefore trigger release of both  $K^+$  and anions<sup>20</sup>, providing a mechanism for the  $Ca^{2+}$  requisite for closure of stomatal pores<sup>10-12</sup>.

The close correlations between  $[Ca^{2+}]_{\text{cyt}}$ -dependent ion channels and the effects of  $Ca^{2+}$  on stomata suggest that the  $Ca^{2+}$ -dependent mechanisms reported here may provide a molecular basis for the regulation of ion transport during stomatal opening and closing.  $I_{K^+, \text{out}}$  channels<sup>24-27</sup> and, more recently,  $I_{K^+, \text{in}}$  channels<sup>27</sup> with similar properties to those described in guard cells, have been characterized in other higher plant cells (for review, see refs 28, 29). Studies of effects of cytosolic  $Ca^{2+}$  on ion channels in these cells may increase our general understanding of  $Ca^{2+}$ -dependent osmoregulation in higher plant cells. □

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## Separate lineages of T cells expressing the $\alpha\beta$ and $\gamma\delta$ receptors

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THE T-cell antigen receptor is a heterodimer molecule composed of either  $\alpha\beta$  or  $\gamma\delta$  chains. The  $\alpha\beta$  receptor molecules are expressed mainly in  $CD4^+ CD8^-$  and  $CD4^- CD8^+$  T cells (helper and killer T cells respectively), whereas the  $\gamma\delta$  receptor molecules are expressed mainly in  $CD4^- CD8^-$  T cells<sup>1-3</sup>.  $CD4^+ CD8^-$  and  $CD4^- CD8^+$  T cells arise from a class of  $CD4^- CD8^-$  T cells during thymus development<sup>4</sup>, raising the question of whether cells

rearranging their  $\gamma\delta$  receptors later give rise to  $\alpha\beta$  T cells further rearrangements of their receptor genes, or whether rearrangements and expression of the receptor genes occur in separate lineages. The  $\delta$ -chain gene is located between the  $V\alpha$  (variable) and  $J\alpha$  (joining) gene segments<sup>5,6</sup> and when the rearrangement allowing  $\alpha$ - and  $\beta$ -receptors occur, the DNA between these segments is deleted as small circles which can be isolated from developing thymocytes<sup>7,8</sup>. The rearrangement status of the  $\delta$ -chain gene in the  $\alpha$ -circles can therefore be investigated to see whether  $\alpha$ -chain and  $\delta$ -chain expression occur in parallel lineages sequentially within a lineage. We find that the  $\delta$ -chain gene in the T-cell receptor  $\alpha$ -circles has a germline configuration, indicating that  $\alpha\beta$  and  $\gamma\delta$  T cells are distinct lineages.

To analyse the  $\delta$ -chain gene rearrangement status in  $\alpha$ -producing T cells, two independent circle libraries were made from the thymuses of AKR mice. The circular DNA isolate was extensively purified (see legend to Table 1), cut with *Eco*RI and cloned into the  $\lambda$  vectors. We obtained  $5 \times 10^4$  recombinant and  $5 \times 10^3$  clones for the first and the second libraries respectively.

TABLE 1 Number of clones from circle libraries that hybridized to specific probes

Probes	Library 1	Library 2
<i>Ja</i> 26	0	0
<i>Ja</i> 27	>300	>100
<i>Ja</i> 19	55	ND
<i>Ja</i> 57	45	ND
A	>500	ND
B	>500	48
C	>500	46
A <sup>+</sup> B <sup>+</sup> C <sup>+</sup>	>500	—
B <sup>+</sup> C <sup>+</sup>	—	46
A <sup>+</sup> C <sup>+</sup>	2	ND
B <sup>+</sup> C <sup>+</sup>	ND	2

Several *Ja* oligonucleotides (*Ja*19, *Ja*57, *Ja*26 and *Ja*27)<sup>9</sup> were used as probes and hybridized to the circle libraries. The *Ja*26 gene segment the most  $\alpha$ -proximal, located 3 kb 5' of *Ca*. The *Ja*19 and *Ja*57 gene segments are located on one *Eco*RI fragment at 41 kb and 38 kb 5' of *C* respectively. The *Ja*27 gene segment is located 60 kb from *Ca* (ref. 9). The oligonucleotide sequences are: *Ja*19, ACCAGCTGAAGGTTA; *Ja*57, AACTTTACAAGTGCAAC; *Ja*26, ACCCAAGTGACGGTAAT; *Ja*2 GCTGACAGTGAGCTCAT. Prehybridization was at 65 °C for 5 h in  $6 \times$  SS 5 × Denhardt's solution, 0.1% SDS and 250  $\mu$ g ml<sup>-1</sup> of denatured salmon sperm DNA. Hybridization was at 37 °C overnight in the above-mentioned buffer containing oligonucleotide probe. Washing was at 37 °C for 2 h with several changes. Circle libraries were prepared essentially as described Okazaki *et al.*<sup>8</sup> Briefly, thymuses from 50 4-5-week-old mice were taken and washed in PBS buffer. The thymocytes were then resuspended in 20 ml nuclear homogenization buffer (0.3 M sucrose, 20 mM Tris-HCl, pH 7.6, 10 mM KCl, 0.5 mM EDTA, 3 mM CaCl<sub>2</sub>) and homogenized with a type B pestle in Dounce homogenizer for <10 strokes. NP40 was added to a final concentration of 0.5% and cells were homogenized again for 7 strokes, or until nuclear breakage was complete. The nuclei were then collected by centrifugation at 2,000 r.p.m. for 5 min and washed 3 times with the same homogenization buffer (30 ml) and 0.5% NP40. The final nuclear pellet was resuspended in 20 ml homogenization buffer, to which 800  $\mu$ l 0.5 M EDTA, 400  $\mu$ l Triton X-100 and 400  $\mu$ l 20% Sarkosyl were added, and the mixture incubated for an additional 15 min on ice. The supernatant obtained after 30 min centrifugation in the SW41 rotor at 40,000 r.p.m. was poured off carefully and phenol-extracted. After extensive dialysis against 10 mM Tris-HCl, pH 7.6, 1 mM EDTA and 100 mM NaCl, the resulting material was banded in a CsCl/ethidium bromide gradient in the presence of 50  $\mu$ g intact pUCPv plasmid DNA as a carrier (pUCPvull is pUC18 plasmid with the small *Pvu*II fragment removed, hence the plasmid does not contain any polylinker sequences and cannot be cloned in an *Eco*RI-cloning vector). Supercoiled DNA was collected and banded one more time. The supercoiled DNA was then extracted with butanol and ethanol-precipitated. Any residual linear DNA was digested with an ATP-dependent DNase I, leaving the supercoiled DNA intact. For cloning, the final preparation of DNA was cut with *Eco*RI and packaged into a  $\lambda$ gt7 vector or  $\lambda$ Zap vector (Stratagene).

ND, Not determined.



To show that the libraries consisted mainly of clones from circular DNA molecules, four  $J\alpha$  oligonucleotide probes specific for the non-conserved portion of the  $J\alpha$  gene and located at different distances from  $C\alpha$  (constant) were used in a hybridization analysis. If the library derived from circles formed during proper rearrangements, the most  $C\alpha$ -proximal  $J\alpha$  segment should be absent from the library, and representation should ideally increase with 5' distance from  $C\alpha$ . As predicted, the probe for the most  $C\alpha$ -proximal gene segment,  $J\alpha 26$  (ref. 9), did not hybridize to any library clone (Table 1). By contrast, the  $J\alpha 27$  oligonucleotide hybridized to several hundred clones in both libraries. Indeed, the unusually high frequency of  $J\alpha 27$ -positive clones indicated that we had cloned circular DNA containing an over-representation of the T cell receptor  $J\alpha$  region. Because we used *EcoRI* as the restriction enzyme to cut the circular DNA during the cloning process, hybridization with the probes for the regions  $J\alpha 19$  and  $J\alpha 57$  located within a single *EcoRI* restriction fragment was particularly informative in assessing the status of the libraries. In the first circle library, 55 clones hybridized to the  $J\alpha 19$  probe, whereas 45 hybridized to  $J\alpha 57$  (Table 1). All 45  $J\alpha 57^+$  clones were also  $J\alpha 19^+$ , indicating that they contained the *EcoRI* fragment of the  $\alpha$ -chain circle in the germline configuration. Because the  $J\alpha 19$  gene segment is located 5' to the  $J\alpha 57$  gene segment, the rest of the  $J\alpha 19^+$  clones, which were  $J\alpha 57^-$ , presumably contained the  $\alpha$ -chain circles from a rearrangement between the two  $J\alpha$  gene segments. The absence of  $J\alpha 19^-/J\alpha 57^+$  clones indicated once again that there was no chromosomal DNA contaminant in the library.

To obtain probes for the mouse  $\delta$ -chain locus, which is located about 75 kilobases (kb) 5' of the  $C\alpha$  gene segment<sup>5,6</sup>, we used a restriction fragment derived from the most 5' end of the cosmid clone TA28.1 (ref. 9) to isolate further cosmids (Fig. 1). Probes were derived that would hybridize to regions 5' to the  $D\delta 2$  gene segment (probe A), within the  $D\delta 2$ - $J\delta 1$  intron (probe B) and 3' of the  $J\delta 1$  gene segment (probe C) (Fig. 1). Because all three probes are contained in a single 7.4-kb *EcoRI* restriction fragment, we anticipated that clones from the  $\alpha$ -chain circles containing the germline configuration of the  $\delta$ -chain would hybridize to all three probes, whereas clones containing rearranged  $\delta$  segments would hybridize to a subset of the probes, except in the case of a rearrangement to the  $J\delta 2$  gene segment, which would give no fragment.

Each of the probes we tested hybridized to >500 clones in the larger library and to 48 clones in the smaller. The number of  $J\delta 1$ -positive clones was in agreement with what would be expected from a circle library with many  $J\alpha$  positives, because a  $J\delta$  probe will detect all the  $\alpha$ -circles and a single  $J\alpha$  probe will only detect a proportion of the  $\alpha$ -circles. (Okazaki and Sakano<sup>10</sup> have earlier reported analysing a circle library in which they detected only 5  $J\delta$  positives and >200  $\alpha$ -positives with a single  $J\alpha$  probe. This apparent conflict with our results may reflect the loss of  $J\delta 1$  clones during the amplification step used in the construction of their library. Our libraries were not amplified at the time of the analysis). All clones that hybridized

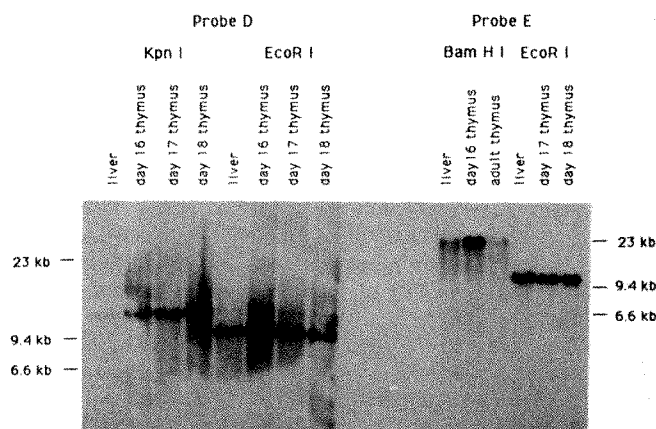


FIG. 2 Southern genomic blots of mouse liver DNA and various mouse thymus DNAs, using the probes D and E depicted in Fig. 1. Probes D and E hybridized to thymus DNAs from days 16, 17 and 18 of gestation, and from adult mouse. These DNAs were digested with several different restriction enzymes spanning the  $C\delta$ - $J\alpha 1$  region (*EcoRI* and *KpnI* for probe D, and *EcoRI* and *BamHI* for probe E, see Fig. 1). This allowed us to detect any moderately frequent rearrangement into the region between the  $C\delta$  and  $J\alpha 1$  gene segments.

to probe C also hybridized to probes A and B (Table 1). There were also a few clones scoring  $A^+C^-$  (these were isolated in early screening and were not tested with the B probe) and  $B^+C^-$  (not tested with the A probe). We isolated several  $A^+B^+C^+$  clones whose restriction fragments were indistinguishable from the germline structure presented by cosmid clone TA9. Although this result strongly implies that the  $\alpha$ -circles have a germline  $\delta$ -chain gene configuration, there are two types of circle arising from  $\delta$ -chain gene rearrangements that could also score as positives: rearrangements to  $J\delta 1$  and  $VD\delta 2$  rearrangements would give  $A^+B^+C^-$  ( $VJ\delta 1$  or  $VD\delta 1J\delta 1$  circles),  $A^+B^+C^-$  ( $D\delta 2J\delta 1$  or  $VD\delta 2J\delta 1$  circles) or  $A^+B^+C^-$  ( $VD\delta 2$  or  $VD\delta 2J\delta 1$  circles) and rearrangements to the  $J\delta 2$  gene segment would give  $A^+B^+C^+$  ( $D\delta 1J\delta 2$  or  $VJ\delta 2$  circles) or  $A^+B^+C^+$  ( $D\delta 2J\delta 2$  or  $VD\delta 2J\delta 2$  circles). The small number of  $C^-$  cases in our library (Table 1), however, in conjunction with the fact that the number of rearrangements to  $J\delta 2$  was at most 1/10 of those to  $J\delta 1$  (ref. 6), indicated that most  $A^+B^+C^+$  clones were derived from  $\alpha$ -circles. We conclude that the  $\delta$ -chain gene is in the germline configuration in the circle products of the  $\alpha$ -chain gene rearrangement.

To determine whether a rearrangement analogous to the T-early- $\alpha$  rearrangement—which takes place between a region 5' to the  $D\delta 2$  gene and a  $\psi J\alpha$  region several kb upstream of the most 5' of the  $J\alpha$  gene segments of the human  $\alpha/\delta$  gene locus<sup>11,12</sup> also occurs in the mouse  $\alpha/\delta$  locus, we performed Southern genomic blots of mouse thymus DNA using probes lying immediately 5' of the  $J\alpha 1$  gene segment (probes D and E in

FIG. 1 Schematic diagram of the T-cell antigen receptor  $\alpha$ -chain and  $\delta$ -chain gene locus. TA3 and TA9 were obtained using a restriction fragment from the most 5' end of the TA28.1 cosmid clone<sup>9</sup> to screen an AKR cosmid library constructed in the pTL5 vector<sup>14</sup>. A plus sign indicates the presence of additional restriction enzyme sites. Probes A, B and C were derived from a subclone containing the 7.4-kb *EcoRI* fragment of the cosmid clone TA9. Probe A was a 1-kb *XbaI/PvuII* fragment, probe B was a 360-base pair *HindIII/PvuII* fragment and probe C was a 2-kb *SacI* fragment. Probes D and E were derived from the cosmid clone TA28.1. Probe D was a 2-kb *KpnI/HindIII* fragment and probe E was a 1.2-kb *EcoRI/BglII* fragment.

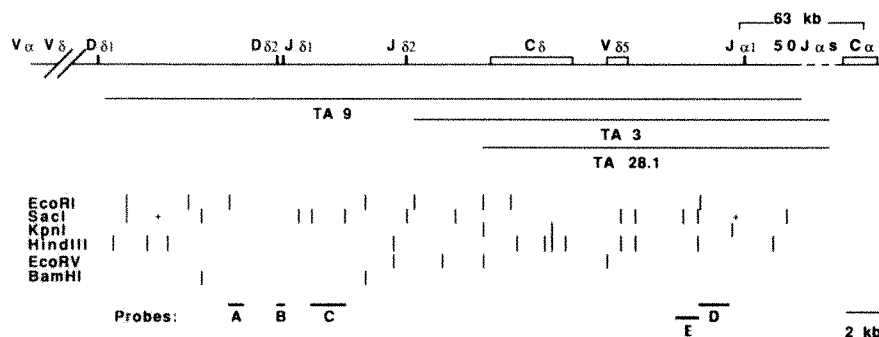


Fig. 1). Probes D and E, however, both detected only the germline bands and no rearranged fragments, even after the blots were exposed for a long time (Fig. 2). In contrast to the human T-early- $\alpha$  rearrangements, which were readily detectable in a Southern genomic blot of human thymus DNA, we found no analogous rearrangement in the mouse and so conclude that the majority of the circle DNAs we analysed came from the  $\alpha$ -chain rearrangement products.

Our results help to clarify the scheme of T-cell differentiation. It has previously been suggested that progenitor thymocytes initially attempt to rearrange the genes for  $\gamma$ - and  $\delta$ -chains; when they fail to produce functional  $\gamma\delta$  receptors, the cells then rearrange their  $\alpha$ - and  $\beta$ -chain genes<sup>13</sup>. This model is inconsistent with our analysis of the  $\delta$ -chain gene rearrangement status in the  $\alpha$ -circles, which indicates that a majority of  $\alpha$ -producing T cells have never rearranged their  $\delta$ -chain locus. Thus T cells expressing  $\alpha\beta$  and  $\gamma\delta$  receptors are not in the same developmental lineage. The split of  $\alpha\beta$ - and  $\gamma\delta$ -producing T-cell lineages could occur after the initial  $\gamma$ -chain gene rearrangements, known to start at day 14 of gestation. Cells with a productive  $\gamma$ -chain gene rearrangement would go on to rearrange their  $\delta$ -chain gene, whereas cells with a non-productive  $\gamma$ -chain gene rearrangement would then differentiate to the  $\alpha\beta$

lineage. Alternatively and perhaps more probably, the T-cell lineage decision would be made independently of the rearrangement process, with one subset of T cells committing to  $\alpha$ -chain rearrangement and another subset to  $\delta$ -chain rearrangement. Rearrangements at the  $\gamma$ -chain gene and the *DJ* of the  $\beta$ -chain gene might then happen in both lineages because of a lack of precise control over these particular events. □

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## ***Distal-less* encodes a homoeodomain protein required for limb development in *Drosophila***

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THE spatial organization of the *Drosophila* embryo depends on the activity of three axial pattern-forming systems. In addition to the anterior-posterior and dorsal-ventral systems that organize the segmented body plan<sup>1</sup>, a proximal-distal pattern-forming system is required to provide positional information for the developing limbs. The development of both the larval and adult limbs depends directly on the activity of the *Distal-less* gene<sup>2,3</sup>. Genetic analysis has shown that *Distal-less* functions as a developmental switch that is required to promote the development of limb structures above the evolutionary ground-state of body wall. Here we provide genetic evidence that indicates a graded requirement for *Distal-less* activity during limb development. Reduction of this activity has a global effect on pattern formation in the limb. The molecular structure of the *Distal-less* locus indicates that the gene encodes a homoeodomain-containing protein which is therefore likely to specify limb development through differential regulation of subordinate genes.

The normal development of *Drosophila* limbs depends on the activity of the *Distal-less* (*Dll*) gene. Several mutations that reduce, but do not completely eliminate, *Dll* activity permit survival to adulthood but cause developmental abnormalities in the adult limbs<sup>3</sup>. Examination of the effects of these alleles on the adult leg shows a progressive pattern of developmental abnormalities that reflects the spatial requirement for *Dll* activity during limb development (Fig. 1). The range of severity of phenotypes caused by different alleles indicates that there is a

graded requirement for *Dll* activity in the developing leg. The most striking feature of these phenotypes is that progressively stronger alleles do not cause deletion of discrete structures in a simple distal to proximal series. Rather, structural elements are lost concomitantly from broad regions of the pattern so that the different regions of the limb are still present but are reduced in size and fused together (Fig. 1*b–d*). These results indicate that reducing *Dll* activity alters the pattern of the developing limb as a whole and suggest that the gene may therefore be responsible for organizing this pattern.

The formal genetic requirement for a graded activity of the gene does not in itself imply that the product need be distributed in a concentration gradient within the developing limb primordium. The ability of individual cells to develop into limb structures, however, depends directly on their own ability to express *Dll*<sup>2</sup>. In this context, the graded requirement for *Dll* expression in individual cells suggests that the concentration of the *Dll* gene product is directly involved in specifying positional information along the proximal-distal axis of the limb.

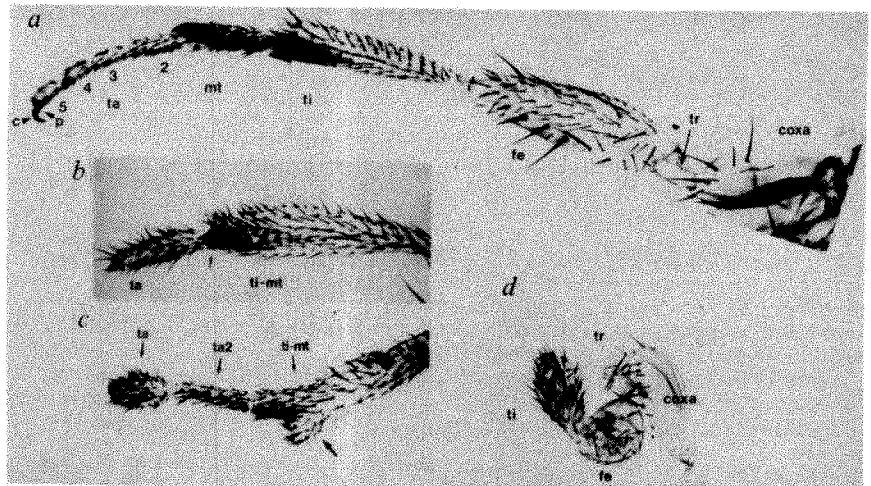
To assess this prediction we undertook a molecular analysis of the *Dll* locus. We cloned the region by chromosomal walking and mapped the molecular lesions associated with eight alleles (Fig. 2*a*). These lesions are evenly distributed through about 38 kilobases (kb) of DNA in which only a single transcription unit, encoding transcripts of ~2.5 and ~3.4 kb, can be detected (Fig. 2*b–d*). Non-contiguous restriction fragments distributed over ~25 kb of the genomic DNA detect the same pair of transcripts. As these transcripts are rare (<10% the level of *engrailed*) we have been unable to isolate full-length complementary DNA clones and can only infer that primary transcripts derived from this relatively large region of genomic DNA are spliced to produce the messenger RNAs detected by the various probes (Fig. 2*e*).

The activity of *Dll* is known to be required during embryogenesis, as amorphic embryos fail to develop larval limbs<sup>2,3</sup>. The precise time during embryogenesis at which the larval and imaginal limb primordia become determined however, is unknown. The vestigial larval limbs are peripheral sense organs<sup>2</sup> which develop during late embryogenesis, following germ band retraction<sup>4</sup>. The putative *Dll* transcripts are first detectable at low levels in post-blastoderm embryos and, as expected, at increased levels during late embryogenesis. Expression of the gene is also required in the developing limb imaginal disk primordia<sup>2</sup>. The transcripts are expressed during the first larva

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FIG. 1 Deletions of pattern elements in the legs of partial loss-of-function *Dll* mutants. *a*, A wild-type male foreleg. Coxa is the most proximal leg segment and develops independently of *Dll* activity<sup>2</sup>. The more distal segments of the appendage are the limb proper<sup>23</sup>. Abbreviations are: tr, trochanter; fe, femur; ti, tibia; mt, metatarsus (also called basitarsus); ta, tarsal segments 2-5; c, claw; p, pulvillus. In the weakest alleles (P and M, for example) only minor reductions in the tarsal region can be detected (not shown). Alleles of moderate severity (IB, 6, 7, 8) cause more extreme reduction of tarsal structures and fusion between tarsal and tibial elements. *b*, Foreleg of a male homozygous for *Dll*<sup>IB</sup>. The metatarsus and tibia are fused. The sex comb of the metatarsus (arrow) and the transverse rows of bristles normally found on the tibia are now found on a common segment (ti-mt), and are both reduced. A single nondescript tarsal segment remains distally. *c*, The leg of a *Dll*<sup>7</sup> homozygote. Note the fused metatarsus and tibia (ti-mt). *Dll*<sup>7</sup> homozygotes typically have normal second tarsal segment (ta2) and a bulbous distal tarsal blob (ta). *d*, Foreleg of a male of the most extreme viable genotypic combination *Dll*<sup>3</sup>/*Dll*<sup>SA1</sup>. Note the greatly reduced femur and tibia associated with normal trochanter and coxa.

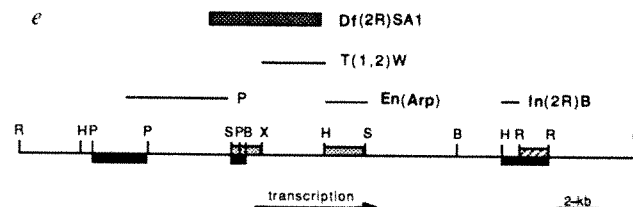
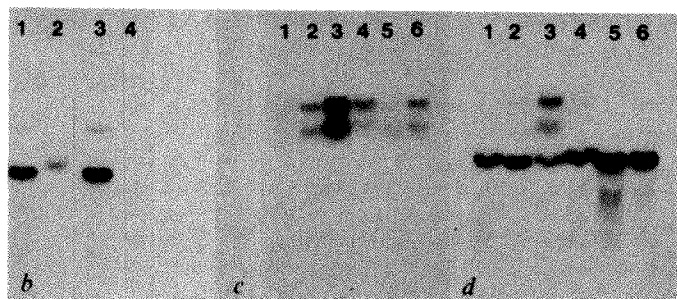
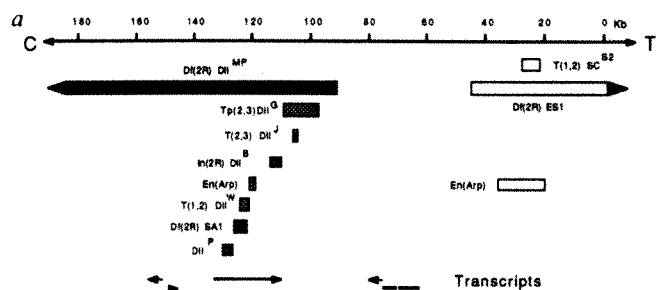
All independent tarsal segments seem to be deleted. In rare cases of a milder genotype *Dll*<sup>3</sup>/*Dll*<sup>3</sup> an occasional sex comb tooth can be detected on the most distal segment, which suggests that this structure may be a fused metatarsus-tibia. Analogous defects also observed in the other segmented appendages of the *Drosophila* adult: the antenna, the maxilla, the labium and



the labrum. Detailed morphological descriptions of the *Dll* hypomorphic phenotypes in these appendages will be presented elsewhere (S.M.C. and G.J., manuscript in preparation).

METHODS. Adult cuticle preparations were as previously described<sup>2</sup>. Some of the *Distal-less* alleles used here have been described previously under the name *Brista*<sup>3</sup>. We have retained the previous allele designations. The origins of the other alleles will be presented elsewhere (S.M.C. and G.J., manuscript in preparation).

FIG. 2 Molecular organization of the *Dll* locus at cytogenetic map position 60E5.6 in chromosome 2R. *a*, The molecular locations of chromosomal rearrangements associated with *Dll* alleles are indicated by filled boxes. The size of the black boxes indicates the extent of the deletions in the alleles MP and SA1. The size of the hatched boxes indicates the uncertainty in the exact location of the breakpoints of the translocations W and J, the transposition G, the insertions P and *En(Arp)* and the inversion B. Open boxes indicate the locations of rearrangements that genetically define a distal limit for the *Dll* locus. *En(Arp)* was generated in a hybrid dysgenic screen (P. Adler) and contains two independent insertions in the region: an insert of 8-kb of DNA associated with the mutation and a P-element near coordinate 30 which lies outside the gene (defined by the breakpoint of *Df(2R)ES1* which is *Dll*<sup>+</sup>). C and T indicate direction of centromere and telomere. The location and orientation (where known) of transcription units within the interval between 60- and 160-kb are indicated. *b*, Reduced stringency hybridization to genomic DNA from *D. melanogaster* (lanes 1, 3) and *D. virilis* (lanes 2, 4) was used to locate coding sequences<sup>24</sup>. The two species are diverged by ~60 million years<sup>25</sup>. Lanes 1 and 2 were probed with the 1.6-kb *SalI*-*XbaI* fragment (stippled box in *e*) located within the DNA removed by the 5.5-kb deletion associated with the SA1 allele. Lanes 3 and 4 were probed with the 2-kb *HindIII*-*SalI* fragment flanking the deletion. Note that although both probe fragments hybridize to the same 6.6-kb genomic *SalI* fragment in the *D. melanogaster* DNA, only the 1.6-kb *XbaI*-*SalI* probe detects a conserved sequence in the *D. virilis* DNA. *c*, Transcription of the *Dll* locus. 10 µg of poly(A)<sup>+</sup> RNA from 0-4 h embryos, 4-8 h embryos, 8-20 h embryos, and first, second and third instar larvae (L1, L2 and L3) in lanes 1-6, respectively, were hybridized with the 1.4-kb *EcoRI* fragment encoding the 3' region of the transcript (indicated by the diagonally striped box in panel *e*). *d*, Same filter as *c*, rehybridized with a tubulin cDNA clone. Note that the apparent concentration of the *Dll* RNAs in the 8-20 h sample under-represents the actual relative level because this lane is underloaded. *e*, Location of the proposed *Dll* exons on the physical map of the locus. Restriction fragments that hybridize strongly to the transcripts in *c* are indicated by filled boxes below the map. B, *Bam*HI; H, *Hind*III; P, *Pst*I; R, *Eco*RI; S, *Sal*I; X, *Xba*I. Within the 870-base pair (bp) *SalI*-*Bam*HI fragment both the 500-bp *SalI*-*Pst*I and the adjacent 370-bp *Pst*I-*Bam*HI subfragments hybridized to the transcript. DNA sequence analysis reveals an open reading frame spanning the *Pst*I site that has the capacity to encode part of a homeodomain. The locations of the transcribed sequences are consistent with the molecular lesions associated with the amorphic alleles. *Dll*<sup>MP</sup> is a large deletion which removes the entire coding region. The small deletion SA1 removes a single exon. The deletion in the SA1 chromosome does not exist in the parental chromosome from which the mutant line was derived and is therefore likely to be the direct cause of the mutant phenotype. The fragments in which the breakpoints of other alleles are located are indicated by lines above the map. The translocation allele W and the inversion B would prevent correct splicing of the mature transcript. The insertion of ~8-kb into the ~13-kb intron in the *En(Arp)* allele also causes an amorphic phenotype. The translocation breakpoint in the allele J is located 3' to the proposed coding region.



METHODS. Molecular breakpoints of the chromosomal rearrangements were determined by *in situ* hybridization to polytene chromosomes using biotinylated probes<sup>26</sup> and by genomic Southern hybridization. The origins and genetic properties of the alleles are described in ref. 2. Reduced-stringency Southern blots were washed in 1 × SSPE at 65 °C. RNAs were fractionated on formaldehyde agarose gels, transferred to nylon and hybridized with <sup>32</sup>P-labelled probes generated by random priming of gel-purified restriction fragments. The orientation of transcription was determined using single-stranded RNA probes and by DNA sequence analysis of short cDNA clones from the 3' ends of the transcripts (not shown).



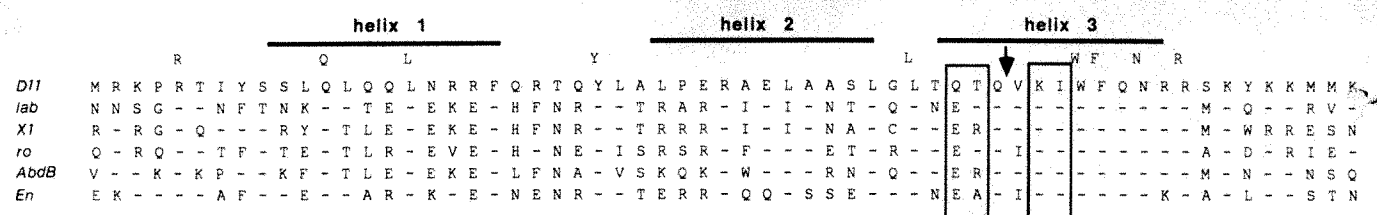


FIG. 3 Sequence comparison of the *Dll* and other homoeodomains. Dashes indicate sequence identity with *Dll*. The positions of the  $\alpha$ -helices determined by NMR analysis of the antennapedia protein<sup>27</sup> are indicated. Boxed residues correspond to solvent-exposed residues involved in sequence-specific contact to the DNA in the bacterial helix-turn-helix proteins<sup>28</sup>. Amino-acid residues conserved in all characterized *Drosophila* homoeodomains are indicated above the *Dll* sequence. The *Dll* HD is diverged from other *Drosophila* HDs with a maximum of 48% amino acid identity to *engrailed*<sup>29,30</sup>, *AbdB*<sup>31</sup> and

*zen2*<sup>32</sup>. In spite of lower overall sequence identity to *labial*<sup>9</sup> (46%) meaningful similarity is greater in that both are interrupted by an intron between residues 44 and 45 (arrow). Other HDs shown for comparison are *rough*<sup>33</sup>, which specifies cell identity in eye development and is like *labial*, and the *Xenopus* *XlHbox1* HD<sup>13</sup>, which may play a role in vertebrate limb development (indicated as XI). Note that this protein, and its newt and human homologues (not shown), are less similar to *Dll* than they are to other *Drosophila* HDs such as *labial*.

instar, at reduced levels during second instar and at higher levels during third instar. The biphasic expression profile is consistent with the requirements for *Dll* activity in development of the larval and adult appendages<sup>2,3</sup>.

The DNA sequences of the genomic fragments that hybridize to the transcripts were determined. A potential protein-coding region was found that has the capacity to encode a polypeptide containing the first 44 amino acids of the homoeodomain (HD), a DNA-binding protein motif found in several developmental regulatory genes of *Drosophila*, yeast and vertebrates<sup>5-7</sup>. The predicted protein sequence diverges significantly from the HD consensus at the position of a potential splice donor site located in the putative DNA-binding helix, and the open reading frame terminates soon thereafter, which suggests that the recognition helix is interrupted by an intron. The remainder of the HD was located in the next more 3' DNA fragment hybridizing to the RNA. This proposed exon was located as an open reading frame following a splice acceptor site which would, if used, complete the HD (Fig. 3). Although we have no direct evidence that the ~13-kb intron is spliced in this way from the *Dll* transcript, the most highly conserved region of most HDs<sup>8</sup> is located 3' to the proposed splice acceptor site. Precedent exists in the case of the *labial* gene of *Drosophila*, which is spliced at precisely the same position in the HD<sup>9</sup>. Several HD-containing proteins bind to DNA in a sequence-specific manner<sup>6,10-16</sup>, and in some cases DNA-binding activity has been shown to reside in the homoeodomain<sup>6,10,16,17</sup>. At present we have too little information about the structural basis for sequence-specific DNA-binding of *Drosophila* HD proteins to assess the significance of the rather divergent amino-acid sequence of the *Dll* HD (Fig. 3).

Recently two other HD-encoding gene products have been shown to exhibit spatial expression patterns consistent with a role for these genes in limb development. The *Xenopus* *XlHbox1* protein (and its murine homologue) are distributed in a concentration gradient along the anterior-posterior axis in the mesoderm of the developing frog and mouse forelimb buds, respectively<sup>18</sup>. The newt homologue of these genes *NvHbox1* is differentially expressed along the proximal-distal axis of the regenerating newt limb<sup>19</sup>. The patterns of expression of these gene products indicate that they play a part in development of the vertebrate limb which is different from that played by *Distal-less* in the *Drosophila* limb. The vertebrate proteins are, in fact, more closely related at the amino-acid sequence level to other *Drosophila* HDs such as *labial* than they are to the *Dll* protein (Fig. 3).

The molecular characteristics of the putative *Dll* gene product are consistent with its proposed genetic function. The graded

requirement for the activity of the *Dll* locus for development of the proximal-distal pattern of the limbs is analogous to the graded requirement for the activity of *bicoid* along the anterior-posterior axis of the *Drosophila* embryo<sup>20</sup>. The graded requirement for *bicoid* activity correlates with a graded distribution of the gene product in the early embryo<sup>21,22</sup>. A graded distribution of the *Dll* protein provides the simplest explanation for the genetic results, although we emphasize that this is not the only possible explanation. If such a gradient exists, the mechanism by which it is established will certainly be different from the simple diffusion mechanism that has been proposed to establish the *bicoid* gradient<sup>21</sup>, as a gradient of the *Dll* protein would have to be established in a field of epithelial cells, rather than in a field of naive nuclei occupying a common cytoplasm. Because the *Dll* protein contains a homoeodomain, it is tempting to speculate that the concentration of the product might, like *bicoid*, directly specify positional information through differential regulation of subordinate genes. □

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# Increased $\gamma$ -globin expression in a nondeletion HPFH mediated by an erythroid-specific DNA-binding factor

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IN man, a shift from  $\gamma$ - to  $\beta$ -globin gene expression in erythroblasts underlies a switch from fetal to adult haemoglobin during development<sup>1</sup>. In hereditary persistence of fetal haemoglobin (HPFH), inappropriately high  $\gamma$ -globin expression in adult life is associated with deletions in the  $\beta$ -globin cluster or with single-base changes upstream of the  $\gamma$ -globin genes. To account for enhanced  $\gamma$ -gene expression in HPFH of the non-deletion type, we tested the nuclear proteins of human erythroleukaemia cells<sup>2</sup> that bind  $\gamma$ -promoter sequences *in vitro* by correlating specific mutations in their binding sites with promoter activity. An erythroid-specific factor (GF-1) binds as a single molecule to the -195 to -170 region and contacts two TATCT(AGATA) motifs, but not the conserved octamer (ATGCAAAT)<sup>3-8</sup> that separates them. We observe that a single change (at -175, T  $\rightarrow$  C) found in HPFH<sup>9,10</sup> leads to increased promoter activity only in erythroid cells. This effect is mediated by GF-1, the human counterpart of the chicken erythroid factor<sup>11</sup> Eryf 1. The form of HPFH we studied here is an inherited disorder which can be ascribed to the action of a cell-specific DNA-binding factor on a mutant promoter.

Using  $\gamma$ -globin-producing human erythroleukaemia (K562) cells, we sought nuclear factors which bind *in vitro* to a -257 to -140  $\gamma$ -promoter fragment that contains the octamer<sup>12,13</sup>, activates a linked  $\beta$ -globin gene on transfer into K562 cells<sup>14</sup>, and is the site of all but one of the single-base changes known to be associated with HPFH<sup>1</sup>. Two types of binding activity were detected (Fig. 1a): one produces gel-retardation complex 2A, is found in all cell lines and is identical to the ubiquitous octamer-binding factor NF-A1/OTF-1; the other gives rise to complexes 1A/1B, is produced only by erythroid cell extracts and is termed GF-1. Binding specificities were distinguished by competition for gel-retardation complexes (Fig. 1b). The core octamer sequence, but not a heterologous sequence or DNA containing the -175 T  $\rightarrow$  C HPFH substitution, inhibited formation of complex 2A. Conversely, complexes 1A/1B were competed out by DNA with the -175 T  $\rightarrow$  C substitution, but not by the core octamer or a heterologous sequence. All complexes were specifically competed out by homologous DNA. Figure 2(a-e) summarizes DNase I footprinting<sup>15</sup> and methylation interference<sup>16</sup> assays of crude and fractionated K562 and HeLa nuclear extracts. Fractions yielding complex 2A (of K562 or HeLa origin) protect and contact nucleotides of the core octamer, consistent with its designation as NF-A1/OTF-1. Fractions containing activity-generating complex 1A/1B protect sequences extending from -195 to -170 and induce hypersensitive sites (Fig. 2a, b). Although the octamer is protected by binding of the erythroid-specific factor, DNA contact sites (Fig. 2c and d) are found only in the flanking sequences.

Several features of the binding of GF-1 to the  $\gamma$ -promoter merit discussion. GF-1 directly contacts repeats of five bases (TATCT or AGATA) in the footprinted region (Fig. 2e). These encompass positions -189 to -185 and -175 to -171. The similarity of DNA contact sites in these repeats suggests two regions that might bind one or two protein molecules. Although two GF-1 molecules could possibly bind to the two motifs in the -195 to -170 region, the following mutation analysis indicates that only a single molecule binds to generate complex 1A.

Complex 1B can be resolved into two species on gels (Fig. 3a): both result from binding of proteolytic fragments of GF-1 (our unpublished data). C  $\rightarrow$  A substitutions at contact sites in either motif (-186 or -172) abolish one or other of the two 1B species. These mutations reduce, but do not abolish, complex 1A. A methylation-interference assay (Fig. 2d) shows complete interference of both motifs in complex 1A. If complex 1A contained two bound GF-1 molecules, either mutation (-186 or -172) would be expected to completely abolish complex 1A and also to generate a faster migrating complex, which is not observed. Furthermore, gel-retardation with probes containing single binding motifs from other globin promoters and enhancers produce complex 1A as the chief species (not shown). A double mutation at -186/-172 abolishes binding to the A $\gamma$ -promoter (Fig. 3b). We conclude that a single GF-1 molecule interacts with a bipartite binding site in the  $\gamma$ -promoter and mutations in either motif can influence formation of the protein-DNA complex.

Evans *et al.*<sup>11</sup> recently described an erythroid-specific globin DNA-binding activity in chickens (Eryf 1) which recognizes a proposed consensus (A/T)GATA(A/G). GF-1 and the independently identified human factor NF-E1 (refs 17 and 18) seem to be the counterpart of this chicken factor. The specific competition for GF-1 binding to the  $\gamma$ -promoter by sequences derived from the 3'- $\beta$ -globin enhancers of chicken or human origin

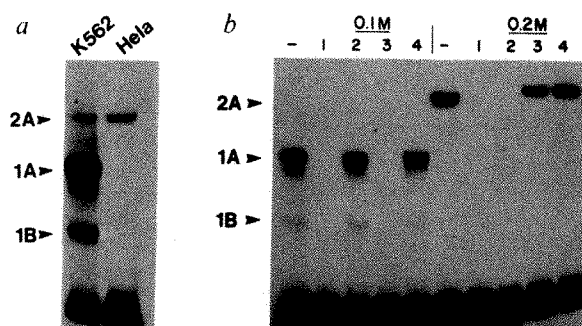


FIG. 1 Detection of DNA-binding activities in K562 and HeLa cell nuclear extracts. a, Gel-retardation assay of crude K562 and HeLa nuclear extracts incubated with a 117-base pair (bp) *HinfI/NcoI* fragment (-257 to -140) of the A $\gamma$ -globin gene<sup>19</sup> promoter. Complexes 1A/1B have been observed only with nuclear extracts of K562, HEL and MEL cells and were absent in human B- and T-lymphoid lines (EBV-line 721 and MOLT 4), monocytic U937 and THP-1, human myeloid HL60 and PLB-985, and murine myeloma cells (P3X) (not shown). b, Competition of gel-retardation complexes by double-stranded oligonucleotides. Heparin-Sepharose-fractionated K562 cell nuclear extract was used in gel-retardation assays with the *HinfI/NcoI* fragment without added competitor (-), or with addition of 20 ng (400-fold excess) double-stranded oligonucleotides (lanes 1-4). The oligonucleotides were as follows: lane 1, a 26-bp  $\gamma$ -promoter sequence from -195 to -169; lane 2, a 32-bp sequence containing four repeats of the ATGCAAAT motif; lane 3, a 26-bp sequence identical to that in lane 1, except for a T  $\rightarrow$  C replacement at -175; and lane 4, a 39-bp sequence extending from -140 to -101 of the  $\gamma$ -promoter, which does not share sequences with the *HinfI/NcoI* fragment. For analysis of complexes 1A and 1B we used a 0.1 M (flow-through) heparin-Sepharose fraction, and a 0.2 M fraction for complex 2A.

**METHODS.** Nuclear extracts were prepared as described<sup>27</sup>. For preparation of K562 extract it is necessary to include leupeptin, pepstatin and aprotinin in addition to phenylmethyl sulphonyl fluoride to inhibit proteolysis. Fractionation on heparin-Sepharose was by loading crude extract in buffer D<sup>27</sup> containing 0.1 M KCl at 4 °C. Step elutions with buffer D containing 0.2 M, 0.3 M, and 1.0 M KCl were carried out after collection of the flow-through (0.1 M). The 117-bp *HinfI/NcoI* fragment of the A $\gamma$ -promoter<sup>19</sup> was 5'-end-labelled and prepared by standard methods<sup>28</sup>. Gel-retardation assays<sup>29,30</sup> were performed on ice in 20  $\mu$ l containing 1  $\mu$ l (0.5-5  $\mu$ g) crude or fractionated extract, 1-5  $\mu$ g poly (di-dC), and 10<sup>4</sup> d.p.m. end-labelled DNA (0.2 ng) in 10 mM HEPES, pH 7.8, 50 mM potassium glutamate, 5 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM dithiothreitol and 5% glycerol. Incubation was for 30 min on ice. Reactions were loaded on to 0.5  $\times$  Tris-borate-EDTA/5% polyacrylamide gels and electrophoresed at 10 V per cm for 2 h at 4 °C.

supports this conclusion (Fig. 3c). In contrast with our results for the  $\gamma$ -promoter, the two Eryf 1 binding sites in the chicken 3'- $\beta$ -globin enhancer act independently<sup>11</sup>. Although the motifs are comparably spaced in the two elements, their orientations are opposite in the chicken  $\beta$ -enhancer, but the same in the  $\gamma$ -promoter. GF-1 binding *in vitro* (Fig. 3c) seems to be dominant over NF-A1 (OTF-1) binding in crude extracts and excludes simultaneous occupancy by this ubiquitous factor on the promoter.

We evaluated the relevance of the binding of two factors *in vitro* with overlapping sequences in the -195 to -170 region to the enhanced expression of  $\gamma$ -globin in HPFH. Promoter activity was assayed in a transient expression system in which wild-type and mutant promoters (encompassing sequences from -385 to +50 of the  $A_\gamma$  gene<sup>19</sup>) linked to the human growth hormone (GH) gene<sup>20</sup> as a reporter were introduced into K562 cells (which synthesize  $\gamma$ - and  $\epsilon$ -, but not  $\beta$ -globins),  $\beta$ -globin-producing mouse erythroleukaemia (MEL) cells, and non-erythroid cells. When introduced into K562 and MEL cells in supercoiled plasmid, the -175 T $\rightarrow$ C HPFH promoter directed production of 4.4 and 4.8 times as much growth hormone as the wild-type respectively ( $P < 0.001$ ). Expression directed by the mutant promoter was not increased in two non-erythroid cell

lines, HeLa and PLB-985<sup>21</sup>. As the -175 T $\rightarrow$ C substitution greatly reduces binding of NF-A1/OTF-1 *in vitro*<sup>17</sup> (Fig. 1), it has been proposed that alleviation of negative regulation by this factor might account for the HPFH phenotype of the -175 T $\rightarrow$ C mutant<sup>12,13</sup>. Evidence against this model was provided by mutant promoters with other substitutions in the octamer. Expression was not enhanced by either a triple substitution (GCA $\rightarrow$ AAG at -180 to -178) or a single-base change (C $\rightarrow$ A at -179) in the octamer. *In vitro* binding to these mutant promoters by the octamer-binding factor, but not by GF-1, is drastically reduced (not shown). The role of GF-1 was investigated by mutating its DNA contact sites flanking the octamer in the context of the wild-type and -175 T $\rightarrow$ C promoters. Introduction of C $\rightarrow$ A substitutions at -186 and -172 or a single C $\rightarrow$ A change at -186 in the -175 T $\rightarrow$ C promoter reduced expression in K562 to the wild-type level. From these transient expression experiments we conclude that (1) the -175 T $\rightarrow$ C HPFH substitution increases promoter strength in erythroid cells of either fetal/embryonic (K562) or adult (MEL) type; (2) the altered expression of the -175 T $\rightarrow$ C promoter in erythroid cells is not due *per se* to reduced binding of a negatively acting octamer-binding factor; and (3) enhanced, erythroid-specific expression of the -175 T $\rightarrow$ C promoter depends on binding of GF-1.

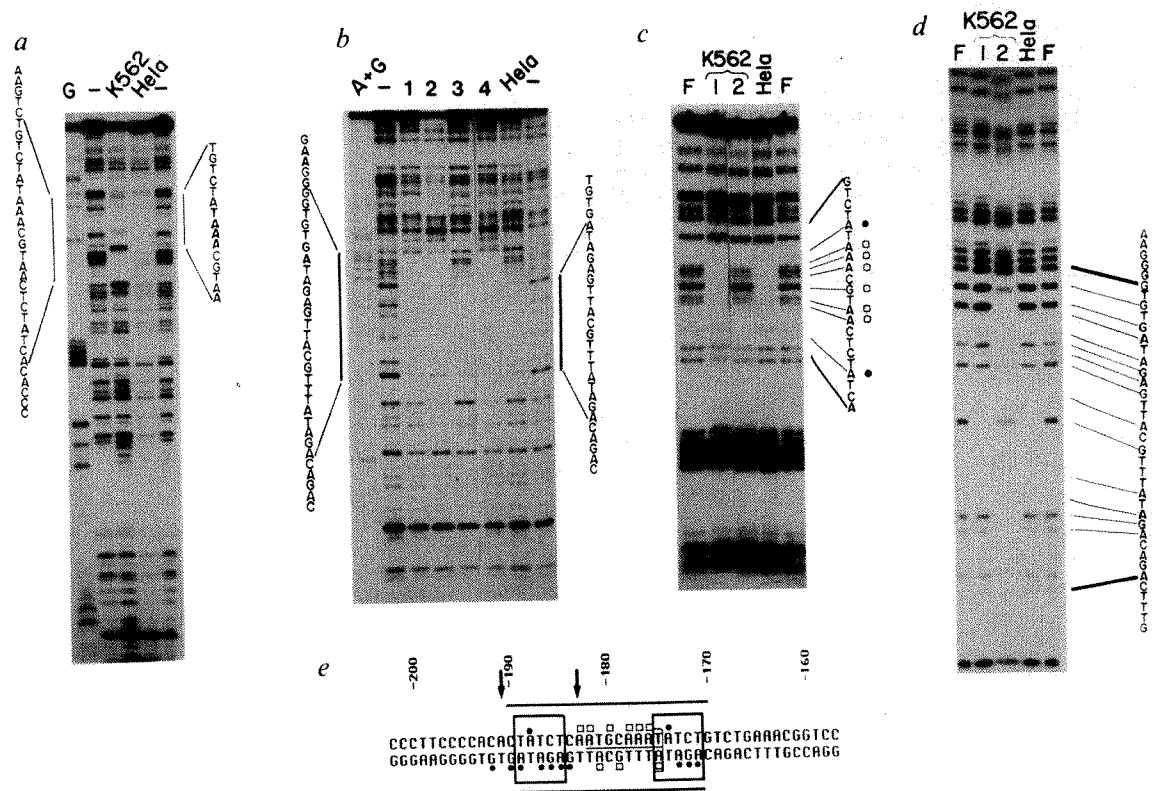


FIG. 2 DNase I footprinting and methylation interference assay of the two DNA-binding activities present in K562 nuclear extracts. *a* and *b*, DNase I footprinting of the HinfI/NcoI  $\gamma$ -globin promoter fragment by K562 and HeLa cell nuclear extracts. Coding (*a*) and non-coding (*b*) strands of the 117-bp fragment were digested with DNase I as described below. Maxam-Gilbert sequencing reactions are shown to the left in each panel. DNase digestions without added protein are denoted as (-). *a*, Crude nuclear extracts of K562 and HeLa are presented. *b*, Crude (lane 1) and heparin-Sepharose fractionated K562 (0.1 M, 0.2 M and 1.0 M fractions, lanes 2-4) extracts and crude HeLa extract. Footprinted areas are denoted by the vertical lines at the left (K562) and right (HeLa) of each panel. For heparin-Sepharose fractionated K562 extracts, DNase I footprints obtained with 0.1 M and 1.0 M fractions are considered equivalent, as we subsequently found that the flow-through (0.1 M) fraction could be quantitatively bound and eluted from the column in the 1.0 M fraction (not shown). *c* and *d*, Methylation interference assays of complexes. G $\rightarrow$ A sequence of unbound (F) fragment DNA is shown for

reference. Interference patterns in K562 complex 2A (lane 1) and complex 1A (lane 2) and HeLa complex 2A (HeLa lane) are shown for the coding (*c*) and non-coding (*d*) strands. Open squares denote methylated bases that interfere with binding in complex 2A; closed circles denote methylated bases interfering with complex 1A. The DNase I footprints and methylation interference DNA contact sites are summarized in *e*. Horizontal lines denote the sequences on each strand protected by K562 extract (total or 0.1 M/1.0 M fractions). Duplicated and contacted TATCT (AGATA) motifs straddling the conserved octamer are boxed. Downward arrows indicate the positions of DNase-hypersensitive sites.

METHODS. DNase I footprinting was as described<sup>15</sup>. Binding was in 50  $\mu$ l containing  $2.5 \times 10^4$  d.p.m. end-labelled DNA, 1-5  $\mu$ g poly (dI-dC), and 5-50  $\mu$ g crude or fractionated nuclear extract in 10 mM HEPES, 1 mM dithiothreitol, pH 7.8, 50 mM potassium glutamate, 5 mM MgCl<sub>2</sub>, 1 mM EDTA, 5% glycerol and 2% polyvinyl alcohol. Methylation interference assays<sup>31</sup> and end-labelled probe methylated<sup>32</sup> were as described.



Whereas enhanced expression seems to require potential GF-1 contact sites, mutations at -186, -172, or -186/-172 revealed no effect on transient expression in the context of the wild-type promoter. As  $\gamma$ -GH constructs have a basal promoter activity which is not cell-type specific, we also assessed the role of GF-1 binding in the -195 to -170 region on the  $\gamma$ -promoter function, using a stable selection assay which exhibits greater tissue-specificity<sup>22</sup>.  $\gamma$ -Promoter/neomycin-resistance gene constructs were introduced into K562 cells and the number of G418-resistant colonies was scored. Consistent with the transient expression data, the -175 T→C/*neo* plasmid yielded 3.1 times more G418-resistant colonies than the wild-type construct ( $P<0.01$ ). C→A mutations at -186/-172 in the presence or absence of the -175 base change reproducibly reduced the frequency of G418-resistant clones 40–50% (relative to wild-type) ( $P<0.05$ ). Additional regulatory elements in the -385 to +50 promoter fragment (outside the -195 to -170 region) thus contribute to its erythroid specificity.

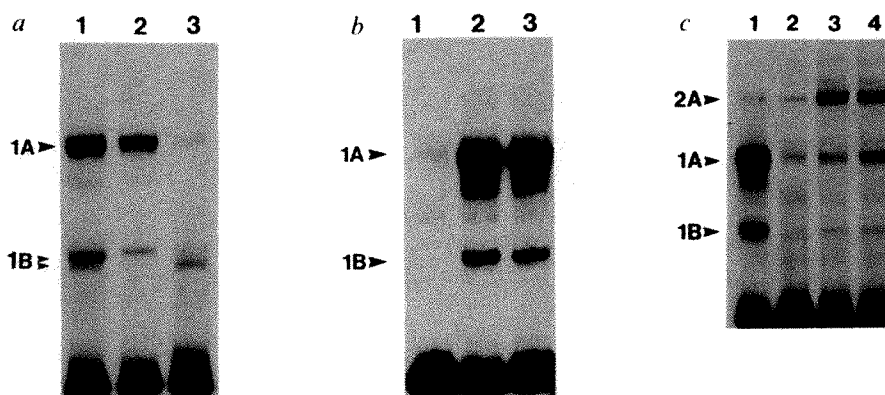
Although GF-1 is implicated in the enhanced activity of the -175 T→C mutant promoter, no major difference in its binding was apparent in the retardation assay (Fig. 3b). It has been suggested that affinity of the -175 T→C mutant promoter for

(presumably) the same erythroid-specific factor is increased 5-fold<sup>17</sup>, but we do not find this. Using either crude or fractionated nuclear extracts, we observe no difference in the affinity of GF-1 for the two promoters (Fig. 4a, b). This finding has recently been supported independently<sup>23</sup>. Although the -175 substitution alters the proximal TATCT motif to CATCT, GF-1 still binds strongly to the proximal motif, as assayed by methylation interference (ref. 17 and our unpublished data). Disruption of the proximal motif, either alone or in combination with a substitution in the octamer (C→A at -172 and C→A at both -172/-179), does not mimic the effect of the -175 T→C mutation on expression. But the DNase I footprint of the mutant promoter shows reduced protection of the proximal motif, which implies an altered recognition of the bipartite site by GF-1 (Fig. 4c). Finally, we have observed that linearization of the -175 T→C promoter/*GH* construct before transient expression in K562 cells largely abolishes enhanced expression.

How then does the -175 T→C mutation lead to enhanced expression? Taken together, our findings suggest that the increased expression characteristic of the HPFH mutant is due to an altered interaction of the mutant promoter with GF-1. Although we find no evidence for a role for the ubiquitous

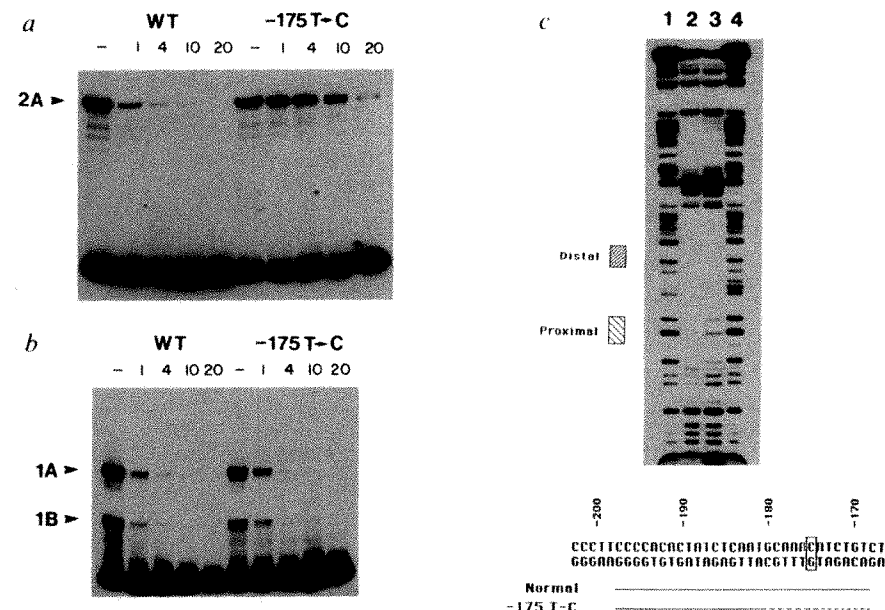
FIG. 3 Binding of GF-1 to mutant promoter DNAs and cross-competition by human and chicken 3'  $\beta$ -enhancer sequences.

a, Gel-retardation assay of K562 nuclear extract with the 117-bp *Hinf*-*Nco*I fragment of promoters containing the normal sequence (lane 1), single C→A substitutions at -186 (lane 2) and -172 (lane 3). b, Gel-retardation assay of K562 nuclear extract (0.1 M heparin-Sepharose fraction) with the 117-bp *Hinf*-*Nco*I fragment of promoters containing T→C, C→A, and C→A substitutions at -175, -186, -172, respectively (lane 1), T→C at -175 (lane 2) and wild-type sequence (lane 3). A double mutant of C→A at -186/172 in the absence of the -175 substitution behaves similarly to the triple mutant (not shown). c, Complexes formed on incubation of the wild-type *Hinf*-*Nco*I fragment with crude K562 nuclear extract (lane 1) were used to compete with ~100-fold excess of double-stranded oligonucleotides: wild-type  $\gamma$ -promoter sequence -193 to -169 (lane 2); human 3'  $\beta$ -globin enhancer TGCTGGCTCCCTATCATGTCCCTTATGGTGC (lane 3); chicken 3'  $\beta$ -enhancer



En4 (lane 4)<sup>11</sup>. Note that competition for GF-1 binding (lanes 3,4) is associated with increased NF-A1/OTF-1 binding.

FIG. 4 Interaction of GF-1 with -175 T→C HPFH DNA. In a and b, the relative affinity of K562 nuclear factors for wild-type and -175 T→C HPFH promoter sequences is compared. Competitions of formation of complex 2A (octamer-binding protein) and complexes 1A/1B (GF-1) are shown in a and b, respectively. Heparin-Sepharose-fractionated K562 extract was used in gel-retardation assays. Complex formation was competed with 0 (-), 1, 4, 10 or 20 ng unlabelled wild-type (WT) or mutant (-175 T→C) oligonucleotide (-193 to -169), as indicated. Note that the -175 T→C mutation reduces the affinity of promoter DNA for the octamer-binding factor by about 20-fold (a), but does not appreciably alter the affinity for GF-1 (b). Competition was similarly observed with larger wild-type and mutant oligonucleotides (-217 to -162) (data not shown). c, Altered binding of the -175 T→C mutant DNA to GF-1. DNase I footprinting was as in Fig. 2, except that GF-1 was purified by affinity chromatography, SDS-polyacrylamide gel electrophoresis, denaturation and renaturation. Purified GF-1 is a polypeptide of relative molecular mass 50,000 (S-F.T. and S.H.O., unpublished data). The non-coding strands of the normal (2) and -175 T→C mutant (3) are presented. DNase digestion without added protein for normal DNA (1) and -175 T→C DNA (4). The positions of the two TATCT motifs are indicated to the left. The extents of the footprints seen with the purified protein on the DNAs are depicted below with a dashed line depicting the reduced protection on mutant DNA. The



-175 mutant sequence is boxed. An additional footprint, present in the -215 region in the vicinity of a low-affinity GATA motif, is seen on both templates with purified factor.

octamer-binding protein in producing the HPFH phenotype, we do not dismiss the possibility that an octamer-binding factor may be involved in  $\gamma$ -gene regulation at some stage *in vivo*. The single base change at -175 alters the interaction of GF-1 with the promoter, rather than simply influencing their mutual affinity as assayed in gel-retardation experiments. The altered footprint of GF-1 on the HPFH mutant DNA could reflect a shift in binding from a proximal (inhibitory) site to a distal (stimulatory) site, but methylation interference demonstrates interaction with both motifs and argues against this simple model. The HPFH mutation seems to activate a region of the  $A_\gamma$ -promoter that normally contributes only a fraction of its overall activity. The effect of the -175 T→C substitution is unique in that other putative HPFH mutations (for example, at positions -202 and -196; refs 24 and 25) do not lead to striking effects on promoter strength in transient K562 or MEL cell assays (our unpublished data).

Binding motifs for GF-1 (Eryf 1, NF-E1) are widely distributed among the regulatory elements of the globin genes<sup>11,17,26</sup>. Evidence presented here and elsewhere<sup>11</sup> establishes a functional role for GF-1 in the human  $\gamma$ -promoter and in the chicken 3'  $\beta$ -enhancer. This factor acts on genes expressed at different developmental stages and, as such, may participate widely in regulation of the globin locus. Our work demonstrates that GF-1 is a positive regulator of  $\gamma$ -gene expression and that a small change in the sequence to which it binds can result in substantially increased promoter activity. If the activity of this factor when bound to DNA is dependent on the specific sequence to which it binds, elucidation of the mechanism responsible for the difference is likely to suggest how an apparently simple binding motif can participate in complex regulated gene expression. Our recent cloning of complementary DNA for GF-1 (manuscript in preparation) will facilitate more direct study of its expression, interactions with regulatory elements, and role in erythroid cell development. □

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## Identification of the long ubiquitin extension as ribosomal protein S27a

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TWO proteins of unknown function are encoded by 3' in-frame extensions of ubiquitin genes<sup>1</sup>. The polypeptides are synthesized as an additional 52 (refs 2-4) or 76-80 (refs 4, 5) amino acids on the C terminus of ubiquitin, an unusual arrangement conserved in man<sup>3,5</sup>, yeast<sup>4</sup> and plants (J. Callis and R. Vierstra, personal communication). Although not homologous to each other or to ubiquitin, both extension proteins are highly basic and contain patterns of cysteine and histidine similar to those proposed to form 'zinc fingers'<sup>6,7</sup>. The longer C-terminal extension protein (CEP80) is 30% lysine and arginine and, when denatured, behaves like a small cationic protein<sup>8</sup>. Its properties after isolation in physiological conditions, however, suggested that CEP80 is part of an RNA-protein complex. Using the antibodies that confirmed the presence of CEP80 in eukaryotic cells<sup>8</sup>, we show here that the protein located on ribosomes. Immunoblotting of rat 40S subunit proteins specifically identifies CEP80 as ribosomal protein S27a.

Native CEP80 binds to DEAE resins unless pretreated with RNase and sediments at 100,000g (K.R., unpublished results). Analysis of a rabbit reticulocyte particulate preparation on sucrose gradients showed that CEP80 was present in fractions containing 80S monosomes and polysomes (Fig. 1a). Dissociation of ribosomal subunits with salt before sedimentation shifted CEP80 to fractions containing the 40S ribosomal subunit (Fig. 1b). In the light of these results, we sought to determine whether CEP80 is one of about 30 proteins known to be components of the eukaryotic small ribosomal subunit.

Like CEP80, most ribosomal proteins are basic, but the long extension is characterized by a lysine/arginine ratio of 3. Inspection of the amino-acid compositions for 33 rat<sup>9,10</sup> and 24 *Artemia* small subunit proteins<sup>11</sup> revealed only four proteins with lysine/arginine ratios of more than 2.5 (Rat S3b, S12 and S27; *Artemia* S27a). S12 from rat is an acidic protein that does not correspond to the extension sequence<sup>12</sup>; S3b is twice the size of CEP80, as judged by SDS-PAGE<sup>10</sup>. Both rat S27 and *Artemia* S27a however, seem similar to CEP80 on the basis of their size and amino-acid composition, as analysed by the method of Cornish-Bowden<sup>13</sup>. Two-dimensional electrophoresis was used to determine whether either of these known ribosomal proteins is the long extension.

S27 and S27a are well separated from other small subunit proteins in the gel system used to name ribosomal proteins<sup>14</sup>, but the protein pattern in these acid-urea gels could only be partially transferred to nitrocellulose. A provisional identification of rat CEP80 was nevertheless possible as the protein detected with CEP80-specific serum was located between and below proteins S28 and S27 (data not shown). This position is occupied by only one known ribosomal protein, S27a<sup>14,15</sup>. Rat ribosomal proteins have been examined in other two-dimensional electrophoresis systems, two of which use SDS-PAGE in the second dimension<sup>15</sup>. These protocols allow the reproducible transfer of proteins to nitrocellulose and, as shown in Fig. 2, our separations of rat 40S subunit proteins are comparable to published patterns<sup>15</sup>. Identification of CEP80 as 40S subunit protein 27a is based on the position of the immunodetected extension as well as its mobility relative to protein S27 in the two electrophoresis systems. S27a is the only small 40S

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subunit protein that has significant shift in relative mobility between the two first-dimension gel systems<sup>15</sup>. CEP80, like S27a, migrates farther than S27 in the low pH system, but not as far as S27 in high pH gels (Fig. 2). These properties identify the long ubiquitin extension as ribosomal protein S27a.

Comparisons of amino-acid contents suggested that rat S27 is the long extension protein, but two-dimensional electrophoresis of 40S subunit proteins in three gel systems identified rat S27a as CEP80. Given these results and the similarity in amino-acid composition of rat S27 and *Artemia* S27a, we conclude that rat S27a was previously misidentified. Chemical modification during isolation may have altered the electrophoretic mobility of the purified rat protein<sup>9</sup>, whereas the isolation of *Artemia* S27a directly from electrophoretic gels would avoid this problem<sup>11</sup>.

Identification of CEP80 as a previously recognized ribosomal protein does not prove that it has a ribosomal function. Co-isolation of the long extension protein with a single ribosomal

subunit reduces the possibility that the association is artefactual<sup>16</sup>, but subunit-specific adventitious binding has been observed previously<sup>17</sup>. CEP80 remains bound to the 40S subunit in salt solutions significantly above the 0.5 M concentration generally considered diagnostic for specific binding. Additional evidence for specificity was obtained by ion-exchange chromatography. Rabbit reticulocyte CEP80 elutes from DEAE columns with proteins that exactly match those of the 40S ribosomal subunit demonstrating that its association with the small subunit is independent of the isolation method (K.R., unpublished results). Further evidence for interaction between the long extension and ribosomes comes from *in vivo* studies of yeast strains lacking the gene for the yeast homologue of CEP80. These yeast grow slowly<sup>18</sup>, have a reduced ratio of small to large ribosomal subunits and inefficiently process the precursor for 18S ribosomal RNA<sup>19</sup>. We expect that a yeast small subunit protein will soon be identified as the 76 amino-acid yeast long-extension protein. Indeed, the deduced amino-acid

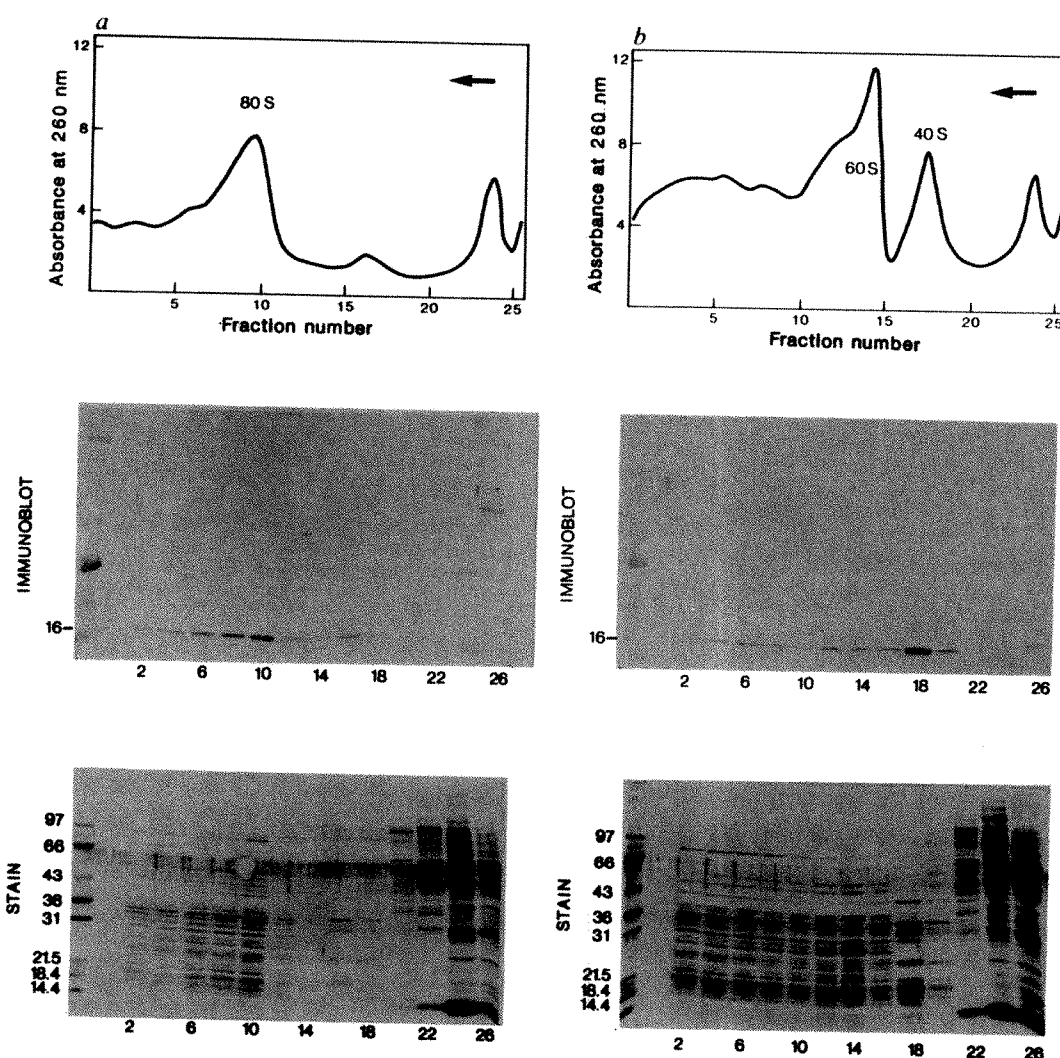


FIG. 1 Sedimentation of rabbit reticulocyte CEP80 in sucrose gradients. Resuspended polysomes were sedimented in low ionic strength buffer (a), or in high salt buffer to dissociate ribosomal subunits (b). Upper panels are profiles of absorption at 260 nm of gradients sedimented in the direction of the arrow. Middle panels are immunostained transfers of proteins from even-numbered fractions after separation by SDS-PAGE. Lower panels are transfers of identical gels stained with amido black. Relative molecular masses were estimated from prestained Rainbow markers (Amersham) on immunoblots and conventional markers<sup>8</sup> on stained blots and are shown on the left, n thousands.

METHODS. Reticulocyte lysate prepared from rabbits<sup>24</sup> was centrifuged for

2 h at 100,000g. Samples of the pellet, containing 40–60  $A_{260}$  units in 0.5 ml, were layered on to 11.5-ml linear gradients of 10–35% sucrose and centrifuged at 187,000g for 2.5 h. Aliquots of the 0.5 ml fractions were analysed by SDS-PAGE on 10–20% acrylamide gradient gels and electroblotted on to nitrocellulose<sup>8</sup>. Immunostaining (using extension protein-specific antiserum P7, goat-anti-rabbit peroxidase-coupled IgG and 4-chloro-1-naphthol) and protein staining were as previously described<sup>8</sup>. The sample and gradient in a were prepared in 50 mM Tris pH 7.8, 12.5 mM  $MgCl_2$ , 80 mM KCl, whereas the sample in b was prepared in the same buffer containing 880 mM KCl and analysed on a gradient containing 500 mM KCl.



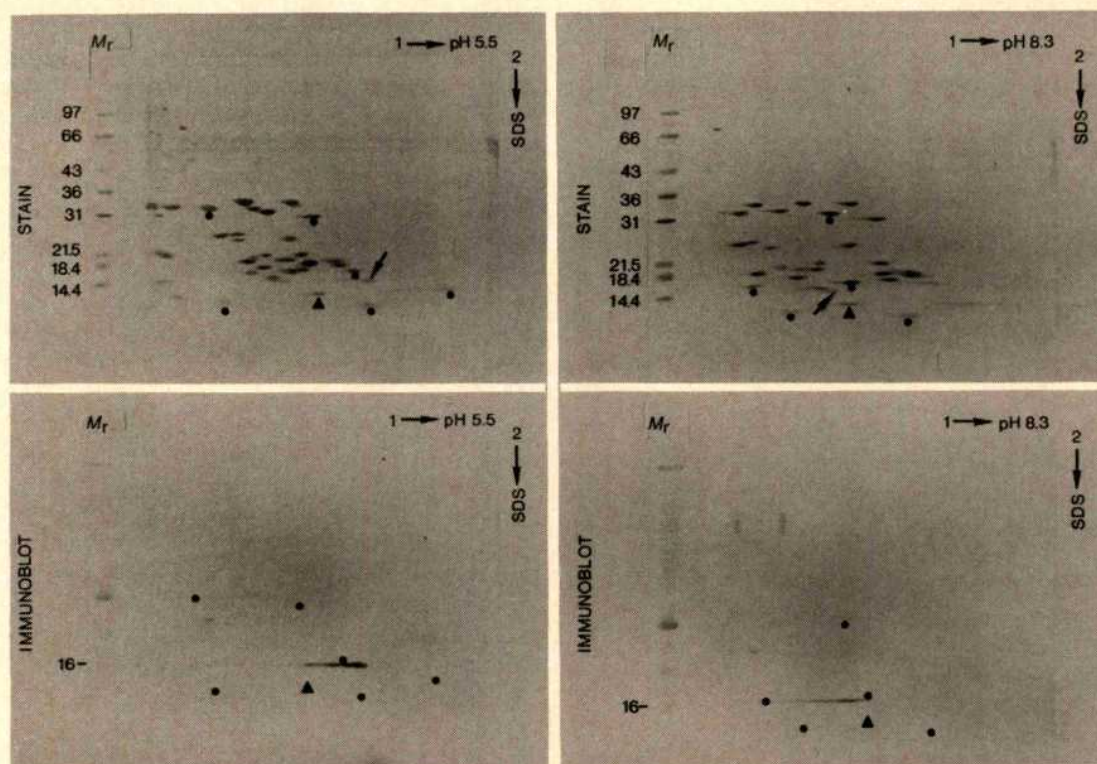


FIG. 2 Immuno-identification of the long ubiquitin extension. Identical nitrocellulose transfers of two-dimensional gels were amido-black stained or immunoblotted after reversible Ponceau S staining<sup>25</sup>. Arrows on the stained transfers indicate the position of the immunoreactive protein. The position of S27 is indicated by a triangle. The relative molecular mass ( $M_r$ ) markers used in Fig. 1 were added to the second-dimension gels (sizes in thousands). METHODS. 40S ribosomal proteins were prepared from the livers of Long-Evans rats as described<sup>15</sup>, and 80  $\mu$ g were separated on acid-urea/SDS

gels (left) or pH 8.3 Tris-borate/SDS gels (right)<sup>26</sup>. SDS-PAGE was performed as described<sup>8</sup>, except that the concentration of Tris in the separating gel was increased to 0.56 M to improve the resolution of small proteins<sup>27</sup>. Transfers were either stained with amido black (upper panels) or Ponceau S to locate individual proteins. After marking the positions of specific proteins on both blots (black dots), the Ponceau S transfers were de-stained and used for the immunodetection of CEP80 as in Fig. 1.

composition of the yeast long extension<sup>4</sup> is nearly identical to that determined for yeast small-subunit protein YS 24 (ref. 20), a protein that is similar in mobility to mammalian CEP80 upon acid-urea/SDS two-dimensional gel electrophoresis<sup>21</sup>.

Why do eukaryotic cells synthesize a ribosomal protein fused to ubiquitin? This novel gene arrangement may have a regulatory role as the translation product is cleaved<sup>8</sup>, and synthesis of the extension protein as a ubiquitin fusion product is not required for wild-type growth in yeast<sup>19</sup>. Degradation of misprocessed, misfolded or otherwise short-lived polypeptides is an important function of ubiquitin-mediated proteolysis<sup>22</sup>. We propose that the fusion gene provides a mechanism to maintain a fixed ratio between ubiquitin, necessary for degrading aberrant proteins, and ribosomes, a major source of aberrant proteins. By binding to ribosomes and its own mRNA, S27a could regulate its own translation and that of ubiquitin. This form of translational regulation has been shown to occur for *Escherichia coli* ribosomal protein operons<sup>23</sup>, but not for the monocistronic genes that encode eukaryotic ribosomal proteins. Although it remains to be seen whether our proposal is correct, conservation of this unusual gene arrangement among eukaryotes implies that there is an important physiological basis for co-synthesis of ubiquitin and a ribosomal protein. □

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# Measuring the new superconductors

J. D. Doss

Faster, more straightforward methods are needed for testing the new high-temperature copper-oxide superconductors. A non-contact eddy-current characterization instrument particularly suitable for screening large numbers of samples will soon be on the market.

SEVENTY-EIGHT years ago in Leiden, the Netherlands, a small team headed by Heike Kamerlingh Onnes discovered superconductivity. The historic measurement on a column of frozen mercury was performed by Onnes's graduate student, Gilles Holst, who nulled a Wheatstone bridge in an attempt to measure a resistance that vanished below 4.2 K. Ever since that landmark measurement, the determination of d.c. resistance has remained the most common method for the characterization of superconductors. The Wheatstone bridge, of course, has now been replaced by sophisticated constant-current sources and microvoltmeters.

Even though d.c. resistance measurements are useful, the typical experimentalist requires additional measurement capabilities for superconductor characterization. Because zero voltage loss occurs only in static fields, other techniques are required to measure the small but finite losses found in the presence of alternating fields. Non-contact techniques are generally preferred (particularly in the study of superconducting thin films) because contacts tend to modify the superconductor surface.

## Magnetic phenomena

With the information available to them, it was quite natural that Onnes and his contemporaries should believe that a vanishing d.c. resistance was the most fundamental indicator of superconducting behaviour. (It is not.) The most definitive property of superconductivity was not discovered until 1933, when Walter Meissner and Robert Ochensfeld demonstrated that magnetic flux is expelled from a superconductor as it is cooled through its transition temperature. The discovery of near-perfect diamagnetism ( $\mathbf{B} = 0$ ) was the first clue that superconductivity is fundamentally a magnetic phenomenon<sup>1</sup>.

The significance of the Meissner-Ochensfeld experiment is not lost on those who are investigating the new family of copper-oxide superconductors. While candidate superconductors may be identified initially through a d.c. resistance measurement, true superconducting behaviour is proven only by demonstration of the Meissner effect. The measurement of magnetization ( $\mathbf{M}$ ) is also a routine procedure in the evaluation of superconductors. Much about the quality of a superconductor can be inferred from its

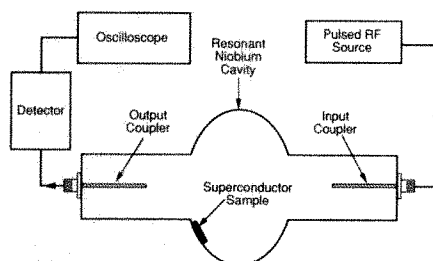


FIG. 1 The surface resistance of a superconducting sample may be determined by measuring its perturbing effect on the  $Q$  of a resonant cavity. The "decrement" method, used for measuring the high values of  $Q$  exhibited by superconducting cavities, is illustrated here.

susceptance ( $\chi$ ); indeed, the superconducting proportion of a multi-phase compound can be determined by measuring the flux expelled from a known volume of material. (Note:  $\chi = M/H$ ;  $\mathbf{B} = \mu_0[\mathbf{H} + \mathbf{M}]$  in SI units, while  $\mathbf{B} = \mathbf{H} + 4\pi\mathbf{M}$  in gaussian units.)

Despite the value of susceptometry, the measurement of flux expulsion is not the most convenient method for screening large numbers of samples, primarily due to cost and time constraints. The magnetic susceptibility of very small samples, such as films whose thickness is less than the London penetration depth ( $\lambda_L$ ), is also very difficult to measure, because they exhibit no significant expulsion of magnetic fields. Thin films are of considerable interest for electronic applications of the new high-temperature superconductors, but other techniques must be used to determine their quality.

## Surface resistance

Determining superconductor energy loss at frequencies ranging up to tens of GHz is important not only for classical radio frequency (r.f.) applications, but also for predicting the performance of superconductors in high-speed computer circuitry. A variety of techniques are employed to characterize superconductor loss at high frequencies, including several approaches where the sample is placed in some type of transmission line: subsequent perturbations are related to the sample's dissipation of energy<sup>2</sup>.

A very effective method for determining the microwave surface resistance ( $R_s$ ) of high-transition temperature (high- $T_c$ ) superconductors involves measuring the perturbation caused by placing the sample inside a resonant cavity. The cavity is first

calibrated by measuring the quality factor ( $Q$ ) of the empty cavity, which is inversely proportional to the resistance of its inner surface, and the  $Q$  of the "loaded" cavity using a sample of similar geometry with a known  $R_s$ , such as stainless steel. With this data, one may determine the surface resistance of a superconducting sample measured in the cavity<sup>3,4</sup>. For example, 3 GHz cavity perturbation measurements on bulk  $\text{Ti}_2\text{Ca}_2\text{Ba}_2\text{Cu}_2\text{O}_m$ ,  $\text{Bi}_2\text{CaSr}_2\text{Cu}_2\text{O}_x$ , and  $\text{YBa}_2\text{Cu}_3\text{O}_x$  yield  $R_s$  values ranging from  $10^{-4} \Omega$  to  $\sim 4 \times 10^{-3} \Omega$ , compared to  $\sim 10^{-6} \Omega$  for Nb (ref. 4).

Cryogenic copper cavities are useful in some instances, but superconducting cavities are generally preferred when higher sensitivity is required. The  $Q$  of non-superconducting cavities may be determined by standard bandwidth measurements, because these have relatively large bandwidths. ( $Q$  is equal to  $f/bw$ , where  $f$  is the resonant frequency, and  $bw$  is the bandwidth within the  $-3$  dB points.) When superconducting cavities are used, the direct determination of bandwidth is not always straightforward, because the  $Q$  of the empty cavity may exceed  $10^6$ . At a resonant frequency of 1 GHz, this implies a bandwidth of only 1 Hz, hardly a value one would wish to measure with a sweep-frequency generator. The higher  $Q$ s are measured by the "decrement" method to determine the fill (or decay) time ( $\tau$ ) of the cavity, which is driven from a pulsed r.f. source (Fig. 1). ( $Q = \pi f \tau = \omega \tau / 2$ , where  $f$  is the microwave frequency (usually near  $f_0$ ) used for the measurement<sup>5</sup>.) While resonant-cavity measurements are the preferred method for determining  $R_s$ , simpler non-contact methods may be used to screen large numbers of samples to identify those specimens which are worthy of more detailed studies.

## Eddy-current techniques

Precision "eddy-current" techniques have been used to determine the resistivity, transition temperature ( $T_c$ ), and penetration depth ( $\lambda_L$ ) of metallic and superconducting samples<sup>6-8</sup>. A modified eddy-current technique may also be used to characterize resistance losses in a qualitative manner, to determine  $T_c$  and the detailed structure of the superconducting phase transition<sup>9</sup>. As illustrated in Fig. 2, coupled inductors  $L_1$  and  $L_2$  may be used to induce r.f. currents in a superconductor

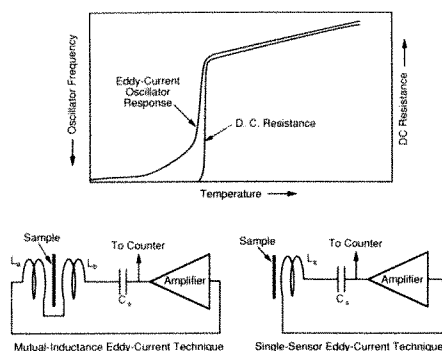


FIG. 2 Eddy-current analysis reveals energy losses below the critical transition temperature usually not apparent from d.c. resistance measurements. In d.c. measurements, a single thread-like superconducting path can "short" the contacts, effectively masking information about behaviour at lower temperatures. Two types of eddy-current oscillator circuits are shown.

sample. The induced current, a function of the sample's energy dissipation modifies not only the mutual inductance, but also the self-inductance of each coil. The inductive sensors are combined with capacitance ( $C_s$ ) to form a series-resonant circuit. The addition of a wide-band amplifier to close the loop yields an oscillator whose frequency is a function of sample energy dissipation (losses).

While the dual-coil system is less sensitive to microphonic noise, very effective systems can also be designed using a single sensor coil. In either case, oscillator frequency is plotted as a function of sample temperature to reveal details about energy losses, yielding information not available from d.c. resistance measurements at temperatures below  $T_c$  (ref. 10). While it is rarely a serious problem, eddy-current characterization does have a limited dynamic range. For extremely high or low values of sample resistivity, changes in resistance may not be sensed for a particular coil-sample configuration. Within limits, the dynamic range can be shifted to a more appropriate region by modifying the coupling between the sample and the sensor coil(s). Los Alamos National Laboratory has transferred its eddy-current technology to Lake Shore Cryotronics, Westerville, Ohio, which is preparing both single-sample and multiple-sample superconductor screening products for the market. □

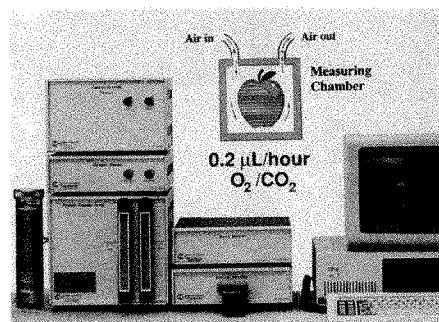
James D. Doss is at the Medium Energy Physics Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA. For more information, fill in reader service number 100.

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## Quantitative aids

A top-loading microbalance, a micro-respirometer, a gaussmeter, and a range of pre-calibrated electrodes are a few of the latest measuring devices for the laboratory.

THE Micro-Oxymax **computerized metabolic computer** from Columbus Instruments measures oxygen consumption and  $\text{CO}_2$  product simultaneously in 10 chambers, varying in size from 100 ml to 10



Micro-Oxymax: the newest respirometer from Columbus Instruments.

litres (Reader Service No. 101). Columbus Instruments says the Micro-Oxymax can be used to measure the metabolism of insects, the proliferation of bacteria or moulds, and the oxygen uptake of tissue cultures and fermenters, with a sensitivity down to  $0.2 \mu\text{L}$ . Measurements can be performed in ambient temperature or in a temperature-controlled waterbath. The measurement intervals can be specified between 5 minutes and 24 hours. The Micro-Oxymax measures the volumes of  $\text{O}_2$  and  $\text{CO}_2$  consumed or produced by the sample under study, and monitors the temperature of the sample chamber. The system is controlled by an IBM-PC or compatible computer; data is saved and reports are generated automatically.

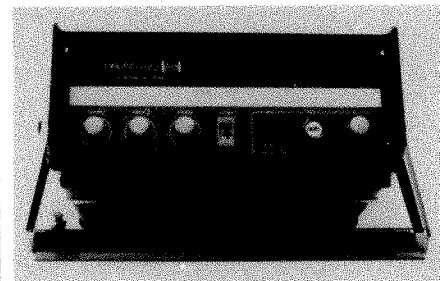
SLM Instruments, Inc. introduced a new **cation measuring spectrofluorometer** earlier this month for use in studies involving intracellular calcium probes such as Fura-2 and Indo-1, and other multiple-wavelength fluorescent probes (Reader Service No. 102). The company's computerized model DMX-1000 Multiparameter Cation Measuring Spectrofluorometer is a combination of a high-speed scanning spectrofluorometer with a light-chopped excitation system. The spectrofluorometer's T-Optics configuration especially suits it for measuring multiple-excitation wavelengths, but it can also be

used for ratiometric measurements and polarization studies. Because of the modular nature of the model DMX-1000, polarizers, filters and other accessories can be installed as needed. Available accessories include a stopped-flow accessory, and a programmable cell changer that can handle four samples.

Genzyme Corporation has launched three new enzyme-based kits for **oligosaccharide analysis** (Reader Service No. 103). Genzyme's UnLinkit-N, AnaLink-N and EndoLink kits can be used to release asparagine-linked oligosaccharides from glycoproteins and glycopeptides, for subsequent analysis using HPLC. Genzyme's kits can be used with the Waters range of columns designed for analysing released oligosaccharides.

### High $T_c$ tech

The French company Drusch has a **nuclear magnetic resonance gaussmeter** for use in solid-state or nuclear physics laboratories (Reader Service No. 104).



The Drusch model RMN 2 nuclear magnetic resonance gaussmeter.

Drusch says the model RMN 2 gaussmeter is simple to operate: the user selects a probe appropriate for the expected field strength, places the probe in the field, and turns on the instrument. Probe alignment is not critical to the measurement, and the probe can be located up to 160 feet away from the readout. The RMN 2 is based on the measurement of the gyromagnetic ratio of protons as the input frequency is swept through the range of interest. Drusch says its gaussmeter can measure any field between 20 mT and 9 T with a precision of  $5 \times 10^{-3}$  mT, and a relative precision of at least  $5 \times 10^{-4}$  mT. The model RMN 2 can also automatically track a varying magnetic field at speeds of up to 10 mT per second.

Mettler's new modular model TA4000 **thermal analysis system** has an extended differential scanning calorimetry range of

-170 to +750 °C, and displays curves in real-time when connected to a PC (*Reader Service No. 105*). The system offers one-key automatic analysis for differential scanning calorimetry, thermomechanical analysis and thermogravimetric analysis. All test programs are permanently stored in the system processor, which can output a complete analysis record to a printer. The system's optional TA72 software allows the model TA4000 to work with an IBM AT or PS/2 computer for on-screen curve plotting, kinetic evaluations, and test data storage.

### Balancing acts

The first **toploading microbalance** is new from Sartorius Instruments (*Reader Service No. 106*). Sartorius's model M3P is sensitive to 1 µg, and has three sensitivity ranges and a maximum capacity of 3 g. Unlike conventional microbalances,



Top-loading microbalance from Sartorius.

Sartorius's balance has no large weighing chamber with mechanical devices to extend and retract the weighing pan. Users can deposit and retrieve samples quickly, with no lockdown procedures to follow or doors to open and close. The microbalance calibrates automatically, and has built-in programs for data processing. The low-profile, compact model M3P has an RS232 interface for connection to a computer for saving or importing data to other programs.

The Japanese company A & D Company, Ltd has an **analytical balance** that comes with a wireless remote control and a vibrating spoon (*Reader Service No. 107*). The company's balance comes in two models: the FR-200 weighs samples up to 210 g in 0.1 mg increments, and the FR-300 handles up to 310 g with the same precision. The balance has a percentage and counting function, and can be used to weigh in grams, carats, pennyweights, decimal ounces, troy ounces, grain units, tolas, taels and mommes. A & D's analytical balance can be calibrated with the touch of a button, and has an interval data output feature that sends weighing data to a computer at set intervals. The balance

controls the vibrating spoon at frequencies between 110 Hz and 230 Hz.

The CP Instrument Company has a **pocket-size digital scale** that measures just 142 × 87 × 15 mm (*Reader Service No. 108*). The top-loading balance is ideal for use in the field, and saves space in the laboratory. Its weighing capabilities belie its small size: the scale measures up to



CP Instrument Co.'s balance fits in a pocket.

500 g in 1-g increments, and up to 1 kg with a precision of 2 g. The balance is described in CP Instrument Company's 160-page catalogue, which has ten pages devoted to balances.

### Spectral studies

Minolta has a compact, **portable spectrophotometer** for colourimetric studies (*Reader Service No. 109*). The model CM-1000 has a flip-up LCD screen, much like a lap-top computer, and a hand-held spectral sensor which measures wavelengths between 400 and 700 nm using a 40-segment silicon diode array and a spectral filter array. The unit has a built-in printer, an RS-232 interface, and a 3.5-inch floppy disk drive. Statistical software allows spectral and colourimetric data to be displayed graphically, in two and three dimensions.

The model ELS-800 **electrophoretic light-scattering spectrophotometer** from Otsuka Electronics was developed for the determination of the surface charges of fine particles and electrophoretic polymers (*Reader Service No. 110*). The system can be used to study protein degeneration, the interaction between proteins and surfactants, the dispersion and aggregation of liposomes and vesicles, and the properties of surfactants and micelles. The model ELS-800 uses the optics of the Heterodyne dynamic scattering method to yield measurements of the zeta potential and electric mobility distribution based on the laser Doppler electrophoretic method. The system's correlator software employs a fast Fourier transform.

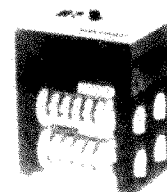
### Image analysis

The BioVision personal **microscope workstation** from Perceptics puts the power of an Apple Macintosh II with a high-resolution colour monitor to work with an optical microscope, an image sensor, a frame grabber, and TCL-Image biological software designed for biomedical research (*Reader Service No. 111*). Perceptics designed the workstation for entry-level image analysis: the company says the mouse, icons, windows and pull-down menus of the Macintosh reduce learning time and simplify operation. The system's frame grabber provides real-time image capture with 256 gray levels. The workstation's TCL-Image software performs arithmetic, linear and non-linear neighbourhood, binary, and morphological operations, along with distance, image and Fourier transformations. Users can also append their own image processing functions, and develop application programs using the existing library functions.

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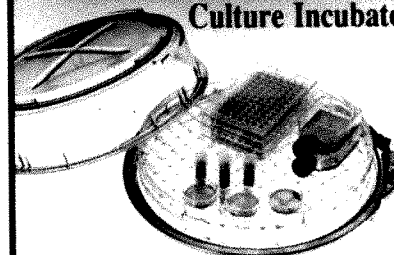


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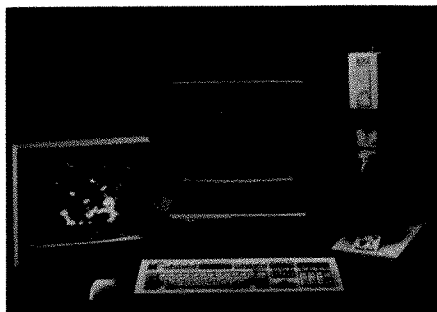
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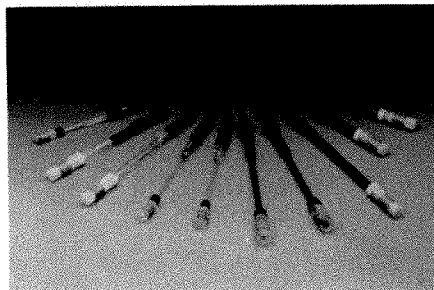
Diamond General's Chemical Microsensor II can be operated simultaneously in the amperometric, potentiometric and thermal sensing modes (*Reader Service No. 113*). The **ion electrode** can determine the concentrations of chemical species such as pH, CO, PO, and PH. The compact Chemical Microsensor II fits easily on



Measure most any ion with the Chemical Microsensor II.

the lab bench, and its battery pack allows it to be carried into the field for performing on-site measurements.

Beckman Instruments has six new **calomel combination electrodes** designed especially for use in biologicals and other silver-sensitive solutions, including Tris buffers, enzyme preparations, tissue culture media, sulfides, bromides and iodides (*Reader Service No. 114*). The Beckman FUTURA Plus electrodes are offered in six styles: with glass bodies measuring 12 mm × 130 mm, 10 mm × 195 mm, 5 mm

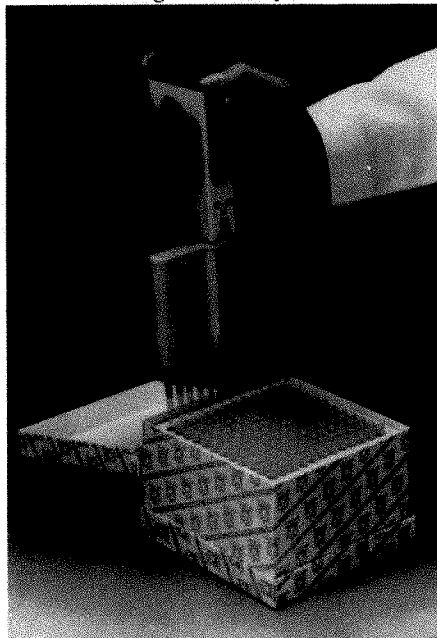


Beckman's array of pre-calibrated electrodes.

× 225 mm, and 5 mm × 178 mm; and with epoxy bodies measuring 12 mm × 130 mm and 7 mm × 225 mm. The electrodes can be used at temperatures of between 23 and 140 °F. Each electrode comes preconditioned in its own Performance Pac vial, and can be used right out of the box, with no soaking.

## Pipette products

Rudolf Brand GmbH has two new **multi-channel pipettes** which can dispense eight or twelve volumes of liquid between 5 µl and 200 µl, at one shot (*Reader Service No. 115*). The Transferpette-8 and Transferpette-12 are ergonomically shaped for comfort, and the volume-setting, pipetting and tip-ejection functions are separated to avoid errors. Brand says the pipettes can be used to fill a large number of microtitre plates without giving the user a cramped hand, because the user's thumb always remains in a natural position. Both pipettes weigh roughly 150 g, and a digital display shows the set volume. The dosing portion of the pipettes can be screwed off for autoclaving. Brand says the Transfer-



Eight- or twelve-channel pipetting from Brand. pipettes function with errors in accuracy of less than 1.5 per cent of the nominal volume.

Flow Laboratories recently launched a cordless, **rechargeable pipetting controller**

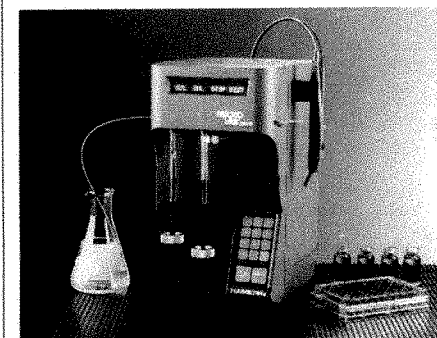
designed for use with pipettes ranging in size from 0.1 ml to 100 ml (*Reader Service No. 116*). Named the Pipetus Akku, the controller weighs 180 g, operates for up to five hours on one charge. When set at minimum power, operation can be extended to 15 hours without recharging. Flow's controller was designed for cell culture media preparation, and comes



Flow's cordless, rechargeable pipette controller.

complete with a recharging unit for £175 (UK). The Pipetus Akku features a 0.2 mm hydrophobic PTFE membrane filter with luerlock connections for air filtration. With normal use, Flow says the unit is maintenance-free, except for periodic filter replacement. Two safety features are included: a safety valve prevents flooding of liquid into the controller, and the entire controller can withstand fumigation with formaldehyde. Dispensing and filling are controlled by separate buttons.

Hamilton's Microlab 1000 **diluter dispenser** has been designed to accommodate liquid volumes of between 1 µl and 25 ml (*Reader Service No. 117*). Users can program the Microlab 1000 with up to 5



The new programmable diluter/dispenser from Hamilton boasts 50 protocols.

protocols by answering a series of yes-or-no questions displayed on the instrument's LED. The diluter/dispenser automatically calculates optimum syringe size, valve size and syringe speed for each application.

*These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.*

**LONDON:** Julie Skeet, 4 Little Essex Street, WC2R 3LF Telephone 01-836 6633 (Telex 262024)  
**NEW YORK:** Marianne S. Ettisch, 65 Bleecker Street, New York, NY 10012 — Telephone (212) 477 9625  
**SAN FRANCISCO:** Megan Van Peebles, Suite 1408, 582 Market Street, San Francisco, CA 94104 (415) 781-3803, 3804 or 3801  
**TORONTO:** Peter Drake, 17 Pine Crescent, Toronto, Ontario M4E 1L1 (416) 690 2423  
**TOKYO:** Phillip Hamill, Shin-Mitsuke Building (4F), 3-6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162-03-267-8751  
**RATES UK** — Rest of world — Display £20 per centimetre. Semi-Display £16.50 per centimetre. Minimum 3 cm. £3 is charged for a box number.

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**CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.**

## Instrumentation Physicist

Daresbury Laboratory operates major national facilities for fundamental and applied research in a wide range of sciences.

### • The Job

We have a vacancy for an Instrumentation Physicist to join a multi-disciplinary research support group with a primary interest in experimental methods in non-crystalline diffraction using synchrotron radiation and related techniques. The successful candidate will be expected to make a major contribution towards the development of specialised instrumentation vital to the research projects of visiting and in-house users embracing a wide range of physical, chemical and biological applications.

The post holder will be expected to solve instrumentation problems which could arise in the control and monitoring of synchrotron beams, X-ray optics, preparation of samples, the dynamics of sample environment, and, in particular, the application of high resolution high rate X-ray imaging detectors.

The post entails working with staff at all levels in varied disciplines and demands qualities of resourcefulness and adaptability.

### • Qualifications and Experience:

Applicants should have a relevant degree or equivalent qualifications. Candidates with a 1st or 2nd class honours degree should have 2 years post-graduate research and development experience. Candidates with other qualifications should have 5 years appropriate experience.

### • Pay:

The appointment will be made at the Higher Scientific Officer grade within a salary range of £10,026 to £13,460 per annum according to qualifications and experience. Further increments are available depending on performance. A non-contributory superannuation scheme and a flexible working hours scheme are in operation at the Laboratory.

**CLOSING DATE:** 14th April, 1989

Application forms may be obtained, quoting reference DL102, from: The Personnel Officer, Daresbury Laboratory, Science and Engineering Research Council, Daresbury, Warrington, Cheshire WA4 4AD.

Telephone (0925) 803467  
(24 hour answering service). (8871)A

**Daresbury**  
SCIENCE & ENGINEERING  
RESEARCH COUNCIL



**ROYAL PERTH  
HOSPITAL**

*'An Equal Opportunity Employer'*

## SENIOR RESEARCH OFFICER

**NH & MRC PROGRAMME GRANT  
Department of Clinical Immunology**

A Senior Research Officer is required to contribute in the area of molecular biology, immunogenetics, receptor biochemistry and cellular immunology. The successful applicant would join a team investigating the immunogenetics of autoimmune disease, with particular attention to the acetylcholine receptor and MHC Class III genes.

Techniques involve monoclonal, antibodies, Southern blotting, DNA sequencing and pulsed field gel electrophoresis. Experience in protein biochemistry would be an advantage.

Appointment will be made initially until the end of 1990 but may be extended to at least four years. Salary would be according to NH & MRC scales, commencing at \$30,737 after six years post Doctoral experience. Supplements may be negotiated.

Written applications addressed to Dr R.L. Dawkins, Department of Clinical Immunology, Royal Perth Hospital, Box X2213, GPO, Perth, WA — Applications should include names and addresses of two professional referees.

**CLOSING DATE:** Friday, 14 April 1989.

(W6006)A

## SEISMOLOGY OR MINERAL PHYSICS UNIVERSITY OF MICHIGAN

The Department of Geological Sciences invites applications for a new tenure-track position at the Assistant Professor level, or in exceptional cases at a higher level, to begin in January 1990 or later. We seek applicants with broad research interests in either seismology or mineral physics for this single open position. We interpret mineral physics broadly, to include both theory and experiment, dealing with both solids and melts. This is an incremental position which augments our existing faculty in these areas. Teaching responsibilities will be at both the undergraduate and graduate level, with occasional participation in introductory earth science courses. A PhD is required. Interested persons should send a résumé, names of five persons from whom the Department may request letters of recommendation, and a brief statement of research interests, to **Prof. Rob Van der Voo, Chairman Search Committee, Department of Geological Sciences, the University of Michigan, Ann Arbor, MI. 48109-1063.** The search will close August 1, 1989, but later applications may be considered. The University of Michigan is an affirmative-action, equal-opportunity employer. (NW3525)A

## DEPARTMENT OF BIOLOGY, QUEEN'S UNIVERSITY KINGSTON, ONTARIO, CANADA

The Department of Biology at Queen's University invites applications for a tenure-track position in the area of **ANIMAL PHYSIOLOGY**. Preference will be given to vertebrate physiologists whose research work involves physiological approaches to environmental or ecological questions, but researchers in all areas are encouraged to apply.

**QUALIFICATIONS:** The successful candidate should be an enthusiastic and competent teacher and will be expected to develop a vigorous research program. Qualifications include a Ph.D. degree and published evidence of excellent research ability.

**EXPECTED DATE OF APPOINTMENT:** This appointment can be effective July 1, 1989 (or as negotiated) and is expected to be at the rank of assistant professor with salary commensurate with qualifications.

**APPLICATION DEADLINE:** The application deadline is **May 15, 1989** or until a suitable candidate is selected. Applications should include a curriculum vitae and a statement of future research interests. Arrangements should also be made to have three letters of reference sent to: **Dr. D. T. Dennis, Head, Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6.**

*In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents.*

Candidates of either sex are equally encouraged to apply. Queen's University is willing to help the spouse of a new appointee to seek employment. (NW3521)A

**British Postgraduate Medical Federation**  
UNIVERSITY OF LONDON



## **Research Co-ordinator Science Planning Unit**

**Salary circa  
£15,000 per annum**

This large Postgraduate Medical School which includes the Institutes of Cancer Research, Child Health, Dental Surgery, Neurology, Ophthalmology and Psychiatry, and the National Heart and Lung and Hunterian Institutes, is establishing a new science Planning Unit to assist in the development and implementation of an academic plan.

The Research Co-ordinator, a graduate preferably with experience in data handling/computing, will be responsible for the day to day running of the project, maintaining close links with Institute staff and the Regional Deans in Medicine and Dentistry.

Further information and application forms are available from Jane M. Jones, Federation Secretary, BPMF, 33 Millman Street, London WC1N 3EJ (Telephone 01-831 6222) to whom applications should be forwarded by 20 April 1989.

(8847)A

## **SYSTEMS NEUROSCIENCE, CELLULAR NEUROSCIENCE, MOLECULAR NEUROSCIENCE; FACULTY POSITIONS DEPARTMENT OF NEUROBIOLOGY AND BEHAVIOR —SUNY STONY BROOK:**

The Department of Neurobiology and Behavior of SUNY Stony Brook is continuing its search for candidates for tenure-track, state-funded positions beginning as early as academic year 1989/90. The Department, which currently consists of 15 members, is undergoing significant redevelopment, and has the resources to grow to 20 members within the next 5 years. Presently available positions are at the Assistant Professor level although an appointment at the rank of associate Professor could be considered. Rank and competitive salaries will be commensurate with qualifications. All applicants should have a minimum of 2 years of postdoctoral experience. Candidates will be expected to develop a vigorous independent research program, and as such ample space and start-up funds will be provided.

Applicants should submit a curriculum vitae, statement of research interests, and names and addresses of three references to: **Dr. Lorne Mendell, Chairman, Department of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY 11794-5230.** SUNY Stony Brook is an affirmative action/equal opportunity educator and employer. AK 60.

(NW3516)A

**AFRC INSTITUTE FOR ANIMAL HEALTH**  
Compton, Newbury, Berkshire. RG16 0NN

## **A Head of Division is required for Immunology and Pathology**

Following a major restructuring of the Institute two major divisions: Molecular Biology, and Immunology and Pathology and two smaller divisions: Environmental Science, and Epidemiology, have been formed. We are seeking to appoint a Head of Division of Immunology and Pathology.

The post will be located at Compton but the successful applicant will be expected to manage an integrated programme across two major sites at Compton and Pirbright and the Neuropathogenesis Unit in Edinburgh. Opportunities exist to work on a number of endemic and exotic diseases of farm animals and to develop fundamental and applied programmes. The Head of Division will plan financial provision, control budgets, encourage collaborative links both within the UK and overseas and take an active role in the management of the Institute.

The successful candidate will have the opportunity to introduce and develop new research topics and appoint to a number of new posts. He/she will be responsible to the Director of Research Professor F. J. Bourne.

Applicants will have a higher degree and broad experience in modern immunological techniques, distinction in a relevant area of immunological research and an ability to manage scientific personnel.

The appointment will be at Unified Grade 5 with a salary on the scale £28,170-£31,602 with opportunity for further, performance related increments to £36,786. There is a non-contributory superannuation scheme.

Application form and further details are available from the **Personnel Officer, AFRC Institute for Animal Health, Compton, Berks, RG16 0NN.**

Closing date: 23 April 1989.

(8861)A

## **ANIMAL BIOTECHNOLOGY CAMBRIDGE LTD SENIOR MOLECULAR BIOLOGIST**

ABC Ltd is a rapidly expanding company in the forefront of the development of advanced transgenesis for the Pharmaceutical, Food and Livestock Industries. We have extensive in-house expertise in animal embryology, *in vitro* culture, stem cell technology and micro-manipulation.

We are looking for an accomplished Molecular Biologist with commercial awareness, to take responsibility for all molecular aspects of our programme and to participate in the development of contracts with our clients.

The successful candidate will have experience in recombinant DNA technology, mammalian gene cloning, and tissue culture. He or she will be responsible for preparing research proposals, setting up projects and managing them. In return we offer a competitive salary and fringe benefits, and the opportunity to work with a well-funded and dedicated team in a stimulating R&D environment.

*Please send your application, including CV and detailed description of previous relevant experience to:*

**Dr Martin Evans**

**Director of Transgenesis**

**Animal Biotechnology Cambridge Ltd  
Animal Research Station**

**307 Huntingdon Road, Cambridge CB3 0JQ**

**Telephone: (0223) 277222 (8860)A**



The security of staple food supplies and incomes in rural African communities is threatened by the presence of the Larger Grain Borer (LGB) insect. Recently introduced by accident into Africa from Central America, it is a highly destructive pest which causes serious losses of maize and cassava in traditional farm stores.

An ODA funded project will carry out work which is designed to obtain a better understanding of the ecology of the LGB and to develop integrated pest management strategies, which are appropriate in the threatened regions of Africa.

## **Entomologist (4 Posts)**

The posts require individuals to work as a member of a multi-disciplinary project team, together with staff of the national research institutes, in identifying and carrying out the work programme. You will also be expected to monitor and control budgets for local project expenditure and to provide a UK based Project Manager with regular reports of technical progress.

### **POSTS ONE and TWO MEXICO**

These appointments are for 2 years from June 1989. The responsibilities of the posts are to carry out a pheromone trapping survey, to determine the distribution of LGB in different regions of Mexico, to assess loss levels sustained by farmers storing maize, to investigate the source of infestation of maize by LGB and to determine the interaction between pest populations in stores and populations of the insect in the wider environment.

Additionally, you will investigate the potential for biological control of the pest in Africa by a specific natural enemy, study the relationship between LGB and other pests of stored maize, assist in identifying aspects of farming and storage systems which may influence the pest status and contribute to the development of sustainable, cost-effective strategies for the control of LGB in Africa.

### **POST THREE KENYA**

A 3 year appointment from June 1989. The responsibilities of this post are to establish a pheromone trapping system which will allow the continued monitoring of changes in the pest's distribution within Kenya, to assess loss levels sustained by farmers during storage, to investigate the source of infestation by LGB and to determine the interaction between pest populations in stores and populations of the insect in the wider environment.

In addition, you will investigate the relationship between LGB, other main pests of stored crops and natural enemies of storage pests which occur in these environments. You will identify and test technologies which can be used at farm level and which will help to reduce losses caused by LGB while recommending sustainable, cost-effective control strategies which can be rapidly implemented.

### **POST FOUR UK (Operating in Kenya/Togo)**

A 3 year appointment from June 1989. Although this post is based in the UK (Slough), you will make frequent trips to Kenya and to Togo in order to work with local research staff. Each trip will depend on specific programme needs, however, you can expect to spend several months overseas each time.

Your responsibility will be to establish and help operate a pheromone trapping system which will allow the continued monitoring of changes in the pest's distribution within Togo and investigate the source of infestation. In addition, you will determine the interaction between populations of the pest on the maize and cassava crops and the interaction between populations of the insect in food stores and those in the wider environment. You will investigate the relationship between LGB and other pests, investigate the bionomics of LGB populations infesting cassava and develop control methods for the protection of dried cassava against attack by the pest.

### **QUALIFICATIONS**

For all posts, applicants should be British Citizens with a postgraduate qualification in entomology or a closely related subject and at least 2 years' experience in a relevant field of work overseas. Ideally, you will have worked with insect pests of stored food grains and you will have been involved in the design and conduct of field and laboratory experiments in this field. For posts one and two, working knowledge of Spanish will be an advantage. For posts three and four, working knowledge of French will be an advantage.

### **TERMS OF APPOINTMENT**

All posts are on contract to the British Government and on loan to the appropriate overseas Government with a UK taxable salary commensurate with age, qualifications and experience. Benefits include tax free overseas allowances, children's education allowances, free accommodation and passages.

For details and application form, quoting the job title and appropriate ref. to: Appointments Officer, Overseas Development Administration AH220, Abercrombie House, Eaglesham Road, EAST KILBRIDE, Glasgow G85 8EA. Or tel. 03552 41199. Ext. 3533.

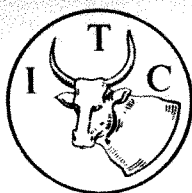
Posts one and two Ref. AH369/MN/N. Posts three and four Ref. AH369/DES/N.



# OVERSEAS DEVELOPMENT

BRITAIN HELPING NATIONS TO HELP THEMSELVES

(8835)A



INTERNATIONAL  
TRYPANOTOLERANCE  
CENTRE

**DIRECTOR**

The Governing Council invites applications for the post of Director of ITC.

ITC is an independent international research Institute committed to the management of trypanotolerance in cattle as a resource for the benefit of livestock owners in the tsetse-infested humid tropics of West and Central Africa. To this end, research programmes have been initiated in production, nutrition including pasture development, trypanosomiasis, helminthiasis, tsetse entomology and genetic improvement. The programmes operate on station and in the surrounding villages with the co-operation of local livestock owners.

Collaborative programmes are in operation with the International Laboratory for Research on Animal Diseases (ILRAD), the International Livestock Center for Africa (ILCA), L'Institut Senegalais de Recherches Agricoles (ISRA), the Overseas Development Administration (ODA) of the British Government, the Centre de Recherches sur les Trypanosomoses Animales (CRTA) and, more recently, with laboratories in Europe and America on a genetic improvement programme which includes both standard breeding and selection and genetic mapping with the aim of identifying the gene or genes responsible for Trypanotolerance.

ITC has three main stations, one near the coast, where the headquarters and main laboratories are located, and two inland, connected by good roads and radio telephone. There are 12 internationally recruited staff and 10 Gambian veterinary and agricultural scientists. They are presently supported by a staff of over 100 and several hundred agricultural workers recruited from the villages. It is intended in the future to extend the regional links with adjoining countries by the establishment of further jointly agreed activities.

ITC was established by Act of The Gambia Parliament 1982. The primary role of the Director will be the co-ordination of the scientific activities of the Centre to enact its aims as laid down in the Act.

Applicants for the Directorship should have a strong scientific background, as well as field experience and a personal interest in cattle. The Director will need to be highly motivated towards the improvement in the quality of village life to which end the research is directed. A working knowledge of English and French is essential. Applicants should hold a Ph.D. or equivalent and have a minimum of ten years experience in research and/or livestock development in tropical Africa, with some experience in leading multidisciplinary teams.

Short-listed candidates will be expected to travel to The Gambia where the final selection for Director will be made. Salary will be by agreement but within internationally accepted scales and according to experience and qualifications.

Further particulars may be obtained from and applications should be made to the office of the Chairman of ITC Council, Professor A.J.S. Davies at the following address: **The Institute of Cancer Research, The Haddow Laboratories, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG, UK. Fax: 01-642 9598.** (8870)A

**UNIVERSITY OF OXFORD  
MEDICAL RESEARCH COUNCIL  
ANATOMICAL NEUROPHARMACOLOGY UNIT  
ELECTROPHYSIOLOGIST**

The Unit is looking for a young electrophysiologist to join an active group working on the microcircuitry of cerebral cortex, using techniques of intracellular recording *in vivo* and *in vitro* combined with light and electron microscopy, immunochemistry, and computer modelling. The successful applicant will find it difficult not to get involved in most, if not all, of these areas. A basic grounding in electrophysiology is important, but most relevant skills will be developed on site. In short, a golden opportunity for the highly-motivated to expand their horizons. The post grade is MRC non-clinical scientific staff (grade 2, short term). Remuneration on the scale for university non-clinical academic staff up to a maximum of £14,500. Tenable for up to 3 years.

The postholder will work with Dr. Kevan A.C. Martin, (tel: 0865 275184), from whom further particulars could be obtained. Applications (CV and names of two referees) to **Prof. A.D. Smith, MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, South Parks Road, Oxford, OX1 3QT. (FAX: 0865 275161).** Closing date 31 May, 1989.

Equal opportunity employer

(8855)A

**UNIVERSITY  
of GUELPH**

**DEAN,  
ONTARIO AGRICULTURAL COLLEGE**

The University of Guelph invites applications and nominations for the position of Dean of the Ontario Agricultural College. The appointment will commence on July 1, 1990.

The University of Guelph has a special responsibility within the Ontario University system to serve agriculture in the broadest sense. The University is committed to a leadership position in education, research and extension related to the use of the components of the agriculture and food system, whether these be physical, financial or human. We have a major concern regarding the impact of resource use on our environment and on society in general.

One of seven Colleges in the University, the Ontario Agricultural College offers a wide range of undergraduate, graduate and research programs in support of agriculture and has a major commitment to international activities. The College has approximately 180 faculty members in nine academic departments; Agricultural Economics and Business, Animal and Poultry Science, Crop Science, Environmental Biology, Food Science, Horticultural Science, Land Resource Science, Rural Extension Studies, and Landscape Architecture. The College currently enrolls approximately 1,300 students in its undergraduate and diploma programs and 460 students in its M.Sc., M.Agr., M.L.A. and Ph.D. graduate programs. The total annual research budget is in excess of \$22 million.

Applicants should have a proven record of leadership and achievement in education and research and a broad understanding of agriculture as it relates to universities, industry, government and society at large. The appointment as Dean will be for a five year term, renewable for an additional five years, and will include a tenured faculty appointment in an appropriate department. Applications and nominations should be submitted by May 15, 1989 to **Dr. J. R. MacDonald, Vice-President, Academic, University of Guelph, Guelph, Ontario, N1G 2W1.**

In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents.

*The University of Guelph is committed to Employment Equity. (NW3513)A*

**Operations Support  
Scientist**

The Synchrotron Radiation Source (SRS) at Daresbury is a national research facility providing high brilliance photon beams at all energies from infra-red to hard X-ray at many different experimental stations.

The Laboratory has an international reputation and is used by industrial and academic scientists from this country and abroad for wide ranging research, including the areas of biological and materials science and surface studies.

The Operations Support Team is responsible for continuous operation of the three electron accelerators which make up the SRS and the provision of front line support for users working on beam lines and experimental stations.

We have a vacancy for an Operations Support Scientist to work on shift as a member of this team, specialising in one or more areas of the experimental programme.

This is a challenging job requiring experience in experimental research work or instrumentation relevant to synchrotron radiation. Practical ability with complex experimental, detector and computer systems is an important requirement for this post.

Minimum academic qualifications of a degree HNC/HND or equivalent are required.

The appointment will be made at Scientific Officer or Higher Scientific Officer level with salary in the range £8,574 to £13,460 depending on qualifications and experience. Further increments are available depending on performance. The Superannuation Scheme is non-contributory.

If you have relevant scientific and technical expertise and you would like to work at a large research facility supporting a varied and expanding research programme, then contact Paul Quinn on 0925 603261 for full details.

Application forms are available from the Personnel Officer, Daresbury Laboratory, Warrington, Cheshire WA4 4AD. Telephone: 0925 603467 (24 hour answering service) quoting reference DL/103.

CLOSING DATE: 13th April 1989.

(8872)A

**Daresbury**  
SCIENCE & ENGINEERING  
RESEARCH COUNCIL

## UNIVERSITY OF OXFORD



### ORGANIC CHEMISTRY LABORATORY

*In association with St. Hilda's College or St. Peter's College*

Applications are invited for a University lectureship in Organic Chemistry tenable with effect from 1 October 1989. Stipend according to age on the scale £9,865-£20,615. The successful candidate may be offered a tutorial Fellowship by St. Hilda's College or St. Peter's College on the terms described in the further particulars. Separate application need not be made for the college appointment. It is hoped to fund this appointment from money made available by the UGC under its new academic appointments scheme (NAAS). Under the conditions of the scheme the successful candidate will normally be near the start of an academic career and under 35. Further particulars of the lectureship and of the college fellowships may be obtained from **Professor J.E. Baldwin, FRS, the Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY**, to whom completed applications (eight typed copies, only one from overseas candidates) should be sent by 29th April 1989. (8857)A

(Re-advertisement)

*The University is an Equal Opportunity Employer*

## UNIVERSITY OF LIVERPOOL

### Leverhulme Centre for Innovative Catalysis

#### University of Dundee

#### Catalysis Research Laboratory

#### Controlled Sulphur Poisoning of Heterogeneous Catalysts

Applications are invited for the post of postdoctoral Senior Research Assistant/Research Associate tenable for up to three years from October 1989. This project concerns the modification of catalytic selectivity by the controlled addition of poisons at levels below those required for total loss of catalytic activity. The project involves two Universities (Liverpool and Dundee), and is sponsored by ICI Chemicals and Polymers Ltd. It is anticipated that the appointee will spend a significant portion of time at each research centre. Techniques of interest include surface analysis and microreactor studies at Liverpool, infrared and Raman spectroscopy at Dundee and catalyst preparation at ICI Chemicals & Polymers Ltd. The appointee will work closely with Dr Graham Hutchings (Liverpool) and Professor Colin Rochester (Dundee) and also maintain close contact with the Catalyst Group, ICI Chemicals & Polymers at Billingham. Informal enquiries may be made to Dr Hutchings, tel. 051-794 3585.

Initial salary within the range £9,865-£10,460 per annum.

Applications, together with the names of three referees, should be received not later than 15th May 1989, by **The Director of Staffing Services (AS), The University, PO Box 147, Liverpool, L69 3BX**, from whom further particulars may be obtained.

Quote ref. RV/304/N.

*An Equal Opportunity Employer* (8841)A

## THE LONDON HOSPITAL MEDICAL COLLEGE

### (University of London)

#### Research in Antibiotic Resistance

Applications are invited from graduates, or those expecting to graduate in Summer 1989, for a post studying aminoglycoside resistance in bacteria. The post is for one year initially, but may be extended for a further year, in which case registration for an MPhil will be encouraged. Remuneration will be at point 1 on the LSO1 scale currently £8,682 p.a. inclusive.

**Applications to: Dr D M Livermore, Department of Medical Microbiology, The London Hospital Medical College, Turner St. London E1 2AD (Tel: 01-377 7644)**

**Closing date 1st May 1989.**

(8848)A

## RESEARCH FELLOW Aquatic Ecology/Toxicology

\$A31,003-\$A45,177

DIVISION OF WATER RESOURCES

GRIFFITH, NSW, AUSTRALIA.

**GENERAL:** The Division's research aims to provide public authorities and the private sector with improved methods for the definition, use and management of Australia's water resources.

The Division's 230 staff are located in Laboratories at Canberra, Perth, Griffith and Adelaide.

One of the programs in the Griffith Laboratory is concerned with the ecology and management of surface water. The program has several projects on water pollution, including degradation of water quality by growth of algae and associated micro-organisms. A close association is maintained with the Murray-Darling Freshwater Research Centre at Albury-Wodonga.

Griffith is situated approximately 400km north of Melbourne and 800km west of Sydney, and has a population of about 15,000. Educational and sporting facilities are excellent.

**THE JOB:** The appointee will complement an existing team working on odoriferous compounds of biological origin, and extend it into a study of toxins in water supplies. Aspects of study could include the eco-physiology of toxin production, development of biological/chemical assay methods; and investigation of options for dealing with the problem, either by modifying the aquatic environment or by conventional water treatment.

The Griffith laboratory is well equipped for chemical analysis (including closed loop stripping, GC-MSD; HPLC; ion chromatography, radioisotope area and autoanalysers). Biological facilities include growth room and glasshouses, SEM, autoclaves and sterile transfer area. The Laboratory is particularly well situated for studies in the Murray Darling Basin, but air links to Sydney and Melbourne also allow ready access to several major urban water storages and systems.

**THE PERSON:** The appointee must have a PhD and demonstrated research experience in a relevant discipline.

A capacity to contribute effectively to a multidisciplinary research team; and to liaise and interact with water managers is highly desirable.

**TENURE:** Appointment is for three years, with prospects for extension to five years. Commonwealth Government Superannuation is available.

**MORE INFORMATION:** Prospective applicants are invited to telephone Dr Kathleen Bowmer (069) 62 0534 for further information. Mrs Shelley McBrien (069) 62 0525 can provide a copy of the detailed job description and selection criteria.

**APPLICATIONS:** Applications should be submitted by 14 April 1989 and quote reference number GR89/2. They should be framed against the selection criteria, and should state relevant personal particulars including details of qualifications and experience. Applicants should nominate at least two professional referees and address their applications to:

**The Chief,  
CSIRO Division of Water Resources  
Private Mail Bag No. 3, Griffith, NSW 2680,  
Australia.**



*CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER* (W6007)A

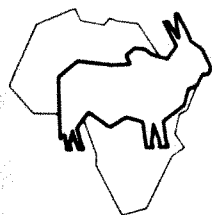
## COLUMBIA UNIVERSITY Department of Anatomy and Cell Biology

Associate Research Scientist to do research on the developing enteric nervous system. Experience with molecular biology of neurotransmitter enzymes, mRNA quantitative analysis, *in situ* hybridization, and receptor neurotransmitter biochemistry required. Send curriculum vitae, statement of research goals, and names of three professional references to: **Taube P. Rothman, Department of Anatomy and Cell Biology, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, New York, NY 10032. EOAAE.** (NW3526)A



INTERNATIONAL  
LIVESTOCK CENTRE  
FOR AFRICA

CENTRE INTERNATIONAL  
POUR L'ELEVAGE  
EN AFRIQUE



## ADDIS ABABA ETHIOPIA

INTERNATIONAL LIVESTOCK CENTRE  
FOR AFRICA  
CATTLE RESEARCH NETWORK COORDINATOR  
VACANCY INT/005/89

### GENERAL

The International Livestock Centre for Africa (ILCA), with headquarters in Addis Ababa, Ethiopia is one of the 13 international agricultural research organizations which are supported by the Consultative Group on International Agricultural Research (CGIAR). The Centre has research activities throughout sub-Saharan Africa and regional offices in Kenya, Nigeria, Niger and Mali.

ILCA's major activities are research, training and information, and its goal is to achieve measurable and sustainable increases in livestock output in sub-Saharan Africa.

With purpose-built offices and research facilities on a large landscaped site close to Addis Ababa's international airport, the Centre's headquarters provide a pleasant working environment and sport and leisure facilities. The city, which enjoys pleasant weather throughout the year, has a large, diverse expatriate community and diplomatic missions from more than 75 nations. It is also the seat of the Organization of African Unity (OAU) and the United Nations Economic Commission for Africa (UNECA). There are several international schools covering American, British, French, German and Italian systems, among others.

### Position

Main duties of the Coordinator, Cattle Research Network will be:

- Plan and coordinate regional and sub-regional research on cattle.
- Guide and assist national scientists in planning and carrying out regional/sub-regional programmes.
- Assist national scientists in the analysis and interpretation of experimental data.
- Organize and run workshops and seminars.
- Solicit material for and edit the Cattle Research Network Newsletter.
- Conduct library searches and assemble a data base on cattle research.
- Prepare the annual programme of work for consideration by the Steering Committees of the Cattle Research Network.
- Prepare an annual report on the year's activities for evaluation by the Steering Committees.

### Qualifications

- A senior scientist in the discipline closely related to cattle research with a minimum of post-graduate training at PhD level and with several years experience in a developing African country. He/She should also have administrative experience.
- Should be able to work effectively with professionals of disciplines within the Cattle Research Network in a multicultural setting.
- Should be able and willing to travel extensively.
- Should be fluent in written and spoken English and/or French but with the ability to learn the other language quickly.

Salary and related benefits are paid in US\$ and will be in line with comparable international organizations. Initial salary will be determined by qualifications and experience.

Applicants should send current curriculum vitae, recent salary history, names and addresses of three professional referees and photocopies of supporting documents (not returnable) before May 30, 1989 in confidence to, the **Personnel Manager, ILCA, P.O. Box 5689, Addis Ababa, Ethiopia.** (W6016)A

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## THE UNIVERSITY OF LEEDS DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY ST JAMES'S HOSPITAL NON-CLINICAL LECTURER (BIOCHEMISTRY)



Applications are invited for the above post.

Salary is on the non-clinical academic scales for Lecturers Grade A (£9260-£14500) or Grade B (£15105-£19310) (under review), according to qualifications and relevant experience.

The applications of biochemistry in the speciality are extremely broad and the applicant will have opportunities for research in almost any aspect of biochemistry. The Department has facilities for most biochemical techniques, including radio-immunoassay, HPLC and electrophoresis. Cooperation and collaboration with other Departments is encouraged and arrangements for access to their facilities and equipment can be arranged. There is significant biochemical work undertaken in other University Clinical Departments at St James's and the University has a large, well-equipped Department of Biochemistry which is very active in collaborative research. The successful applicant will be expected to undertake teaching in the Department and in the Department of Biochemistry.

Informal enquiries about the post may be made to Professor R J Lilford, Department of Obstetrics and Gynaecology, St James's Hospital, Leeds LS9 7TF (Tel (0532) 433144 Ext 5872/4524), Fax (0532) 426496).

Application forms and further particulars may be obtained from and completed applications forwarded to the **Registrar, the University, Leeds LS2 9JT** (Tel (0532) 333963 — direct line), quoting reference number 99/8. Closing date for applications 25 April 1989. (8839)A

## HORSE RACING FORENSIC LABORATORY DIRECTOR

Newmarket

circa £37,500 + car

Applications are invited for the prestigious post of Director of the internationally-renowned Horseracing Forensic Laboratory, Newmarket, which will become vacant in October 1989, on the retirement of Dr Michael Moss, MSc PhD MIBiol CChem FRSC. The Laboratory is a subsidiary of the Horserace Betting Levy Board, a statutory body which provides funds and services to horseracing.

The Laboratory, employing 55 people, provides a drug screening service to the Jockey Club, and also to provide mainly overseas, clients on a commercial basis. It also operates an extensive research programme in support of the detection of prohibited substances in racehorses.

Proven management capability is required, together with the flair, dynamism and experience in relevant scientific disciplines necessary to oversee the cost-effective, increasingly automated testing operation; to direct and motivate a team of skilled research scientists; to advise the Jockey Club on anti-doping policies and Rules and to liaise with their security and veterinary personnel; and to exploit commercial opportunities at the Laboratory as they arise. The Director will be required to keep in close touch with pharmacological developments in this country and overseas and with counterparts in other major racing countries.

The excellent benefits package includes a car and contributory pension scheme. In the first instance, please write with full CV, including current salary, to:

**Martin Crawshaw, Company Secretary, HFL Ltd,  
Horserace Betting Levy Board, 52 Grosvenor Gardens  
London SW1W 0AU. Tel: 01-730 4540 (8874)A**

## UNIVERSITY OF WALES COLLEGE OF MEDICINE INSTITUTE OF MEDICAL GENETICS (Department of Medicine)

### RESEARCH TECHNICIAN

Research Technician (Grade 4) required to work on a Muscular Dystrophy Association (USA) project, "The identification of the Myotonic Dystrophy gene". The work involves the use of recombinant DNA techniques, and candidates should have a degree in a relevant subject.

The appointment will be for one year initially with possible renewal for a further two years. Salary will be within the scale £7,505-£8,688.

Applications in the form of curriculum vitae (quoting Ref. No. M6/219) with names and addresses of two referees should be sent to the **Registrar and Secretary (Personnel Office), University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN** from whom further particulars are available (Tel. 0222/755944 Ext. 2296).

Informal enquiries can be made to Dr. D. J. Shaw (Te. No. 0222/762299).

Closing date: three weeks from the appearance of this advertisement. (8842)A

## MEDICAL RESEARCH COUNCIL

Radiobiology Unit

Chief Research Officer

A permanent position is available in the LRF Radiation Leukaemogenesis Laboratory for an experienced CELL BIOLOGIST to join a group studying the effects of ionising radiation on haemopoiesis. The person appointed will be responsible for cellular and molecular studies of changes associated with the transition from normal to eukaemic haemopoiesis in an experimental model of acute myeloblastic leukaemia. A particular objective would be to develop in-situ hybridisation techniques.

Remuneration will be at an appropriate point on MRC scales: £14,444-£16,897 (under review). An appointment would also be considered at Senior Research Officer level.

The Unit is situated on the main Harwell Laboratory site in pleasant rural surroundings equidistant to Oxford and Newbury. Excellent working conditions and research facilities, pension provision, hotel accommodation available.

Application forms and job details may be obtained from the Personnel Officer, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD. Informal enquiries are welcome to Dr. Eric Wright on 0235 834393 ext 327. Closing date: 21st April 1989.

The Council is an equal opportunity employer. (8854)A



## Research Assistant

Applications are invited for the post of Research Assistant to work on a five-year programme grant funded by the Wellcome Trust involving the structure and function of the genes encoding cell adhesion molecules in muscle and brain. Techniques involved will include mammalian cell culture, gene transfer and associated DNA/RNA analyses (see, e.g. CELL, 50, 1119, 1987; 55, 955, 1988). Experience in cell or molecular biology is preferred but training can be given. This is a responsible position in a highly active research group and should interest goal orientated individuals with interests in Molecular Biology.

Salary up to £13,330 p.a.

Applications, including a full c.v. and the names of two referees, should be sent to Dr Frank S. Walsh, Department of Neurochemistry, Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG. Tel: 01-837 3611 Ext. 4204. Closing date: 15 April 1989.

(8849)A

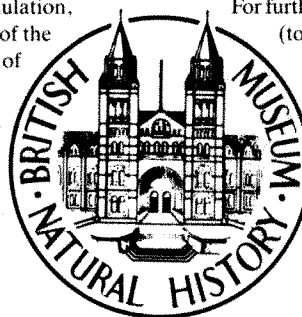
# KEEPER OF BOTANY

## With the vision to guide policy formulation London

The British Museum (Natural History), department of Botany, houses one of the world's largest and most important collections of botanical specimens and is held in high regard both at home and abroad for its research on the relationships, biogeography and evolution of plants.

The Keeper of Botany plays a major policy forming role within the Museum ensuring the effective financial, administrative and resource management of the department together with the leadership of a team of 49 staff including 39 scientists.

Reporting to the Museum Director you will also be responsible for the formulation, maintenance and development of the collection and for the provision of scientific and enquiry services. In addition you will represent the Museum on outside bodies and through active involvement in relevant learned societies.



An equal opportunity employer

As an active researcher with a high professional status you should have demonstrated your work through publications in relevant aspects of botany. Probably with a degree or higher qualification in Botany, you must have a knowledge of the care and maintenance of large scale collections as well as proven management and administrative abilities.

Salaries are in the range £28,170-£31,600 with further increments, depending upon performance, up to £36,785. An additional £1750 London Weighting Allowance is available.

For further details and an application form (to be returned by 28 April 1989) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours). Please quote ref: S/7897.

(8850)A

## HARROW HEALTH AUTHORITY THE KENNEDY GALTON CENTRE FOR CLINICAL GENETICS

Level 8 Clinical Research Centre  
Northwick Park Hospital  
Watford Road  
HARROW  
Middx HA1 3UJ

### PRINCIPAL CLINICAL MOLECULAR GENETICIST

Required to take charge of one of the two Regional DNA Laboratories which provide a diagnostic service to the North West Thames Region. The laboratory has been recently developed in new accommodation in the Centre which has a major role in the provision of genetic services to the Region.

Salary scale: £15,180-£20,989 p.a. plus £757 London Weighting. Whitley Council Conditions of Service.

Informal enquiries welcome: Telephone Dr M Ridler or Dr J Squire on 01-422-8577.

Application form and job description from the Personnel Department, Northwick Park Hospital, Watford Road, Harrow, Middlesex HA1 3UJ. Tel: 01-864-5311 ext. 2410 or 01-423-5370 (24 hours). Ref. P607X (8868)A

## DEVELOPMENTAL BIOLOGY AND NEUROSCIENCE

Two tenure track faculty positions in the Department of Anatomy Program in Cell Biology and Neuroscience. Applicants for the Associate Professor position should have an ongoing extra-murally funded research program, an established record of scholarship and a willingness to direct team-taught and problem-oriented medical gross anatomy; applicants at the Assistant Professor level should have a postdoctoral experience, an active research program, and a willingness to teach a portion of medical gross anatomy. Preferences will be given to cellular/molecular approaches to study CNS development and plasticity and to molecular studies of cell-cell interactions, proto-oncogenes, growth factors and regulation of gene expression. The Medical Center has active programs in molecular genetics, neuroscience, retinal research, aging, and ultrasonic evaluation of arterial disease. A National Biomedical Research Technology Resource Center, including IVEM, TEM, and SEM, is on campus.

Send CV and names of three references to: **Craig K. Henkel, Ph.D. Department of Anatomy, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103 AA/EOE.** (NW3518)A

## UNITED MEDICAL AND DENTAL SCHOOLS LEVERHULME GRANT — RESEARCH ASSISTANT

Applications are invited for a RESEARCH ASSISTANT in the Division of Anatomy, Guy's Campus. This three year post is open to applicants from October 1989, with a first or upper second class degree in human anatomy, anthropology or a related biological science. The primary aim will be to organise, preserve and catalogue a documented human skeletal collection housed in the crypts of St Bride's Church, London. The applicant will have the opportunity to work towards a PhD qualification using the skeletal collection as the basis for their research.

Salary from £8,675 per annum, plus £1,650 London Allowance.

Applications with full CV and names and addresses of two referees or any further information, to Dr J L Scheuer or Dr S M MacLaughlin, Anatomy Department, UMDS, Lambeth Palace Road, London SE1 7EH, 01 928 9292 X2490 quoting ref G/A/318 by 1 May 1989. (8844)A

The World Health Organization (WHO) is an inter-governmental agency internationally recognized for its integrity and lasting achievements. Guided by humanitarian concerns, WHO works to direct and coordinate global and national efforts to improve the health of peoples throughout the world. To meet its objectives, WHO depends on staff members with special qualities of leadership, dedication and commitment.



The Tropical Diseases Research Programme (TDR) has taken a leading role in the research and development of new and improved tools for the prevention, diagnosis and treatment of tropical diseases and now wishes to promote further development, production and testing of these new products, with specific emphasis on their production and testing in developing countries. Such new products, which include drugs, diagnostic tools, vaccines and new tools/methods for vector control, are destined for eventual integration into disease control programmes.

TDR is seeking to hire a scientist/product development manager with experience in the development, production and testing of biotechnological products and pharmaceuticals. The successful candidate will work in close collaboration with various TDR Steering Committees and will provide expertise and support related to the promotion and management of product development, particularly in relation to disease endemic countries. The product development manager will be expected to contribute to TDR's strategies for product development, including the establishment of innovative and productive interactions with industry, and to coordinate their implementation. One of the major challenges will be to initiate the development and production, in particular in developing countries, of biotechnology-derived products for use in large-scale field testing and eventual use in disease control programmes.

Applicants should have extensive experience in biotechnology or pharmaceutical research and development, ideally in a major biotechnology or pharmaceutical company, including up-to-date knowledge of the development, production and testing of biotechnological products and pharmaceuticals. A broad knowledge of the biology of tropical diseases would be an asset. A doctoral level degree in biological sciences is a requirement, as is an excellent knowledge of English or French, with a good working knowledge of the other.

Please send your detailed *curriculum vitae* and a letter of application by 30 April 1989 to **Personnel, WHO, Avenue Appia, CH-1211 Geneva 27, Switzerland, quoting PDM/TDR/89** and the name of the journal in which this announcement appeared. Applications from women are encouraged. Only candidates under serious consideration will be contacted. (W6017)A

*The UNDP/WORLD BANK/WHO special programme for Research and Training in Tropical Diseases (TDR) is a globally coordinated effort to bring the resources of modern science to bear upon the control of major tropical diseases and to strengthen the research capability of endemic, developing countries where new disease control technologies are most needed. To meet its objectives, TDR has recruited into its global network scientists of the highest calibre to participate in this international research effort.*

## TDR

### Scientist/Product Development Manager Tropical Diseases Research Programme WHO Geneva, Switzerland

## UNIVERSITY OF OTAGO Dunedin, New Zealand

### FIXED-TERM LECTURESHIP (SCIENCE) IN BIOCHEMISTRY

Applications are invited from science graduates with appropriate experience for appointment to a fixed-term Lectureship in Biochemistry. The appointment is for a period of 12 months, which is to fill a vacancy created by the absence of a permanent member on leave without pay, not subject to extension or renewal. The post will be available for three years from approximately 1 July 1989.

Salary: NZ\$35,000-NZ\$42,000 per annum.

The appointment, which will be made at a level that is suitable for a recent Ph.D. graduate, is open to applicants with research experience in any branch of Biochemistry, Molecular Biology and would provide experience in a University Department for a graduate who wishes to make a career in academic biochemistry. Extensive postdoctoral experience is not an essential pre-requisite.

Further particulars are available from Appointments (36182) Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF; or from the Registrar, University of Otago, P.O. Box 56, Dunedin, New Zealand. Enquiries may be addressed to the Head of the Department of Biochemistry, Professor G.B. Petersen, Fax: NZ (024) 741 607 or by telephoning: NZ (024) 797 864.

Applications quoting reference number A89/15 close on 1 July 1989.

Equality in Employment opportunity is University Policy. (W6014).

## UNIVERSITY OF LIVERPOOL

### Department of Biochemistry

#### Protease and Proteolysis Research Group

Applications are invited for the post of postdoctoral

### Senior Research Assistant

Funded by the S.E.R.C. Biotechnology Directorate to study the action of metallo-endopeptidases in the synthesis of biologically-important peptides and peptide derivatives. The project will be conducted in collaboration with Dr. R. Cosstick, Department of Chemistry. Informal enquiries may be made to Dr. R. J. Beynon, Tel. 051-794 4359.

The post is tenable immediately for a period of up to three years at an initial salary within the range £9,865 - £11,680 per annum. Applications, together with the names of three referees, should be received as soon as possible by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained.**

Quote ref. RV/308/N.

An Equal Opportunity Employer (8840)A

## UNIVERSITY OF DURHAM

### DEPARTMENT OF GEOLOGICAL SCIENCES

### RESEARCH POST IN SEISMOLOGY

Applicants are invited for the post of Research Assistant or Senior Research Assistant in Earthquake Seismology to be responsible for running a seismic network in the Caribbean. Candidates should preferably have practical experience in seismic acquisition, and research experience in seismology. Consideration will also be given to applicants with a practical electronics background. Informal enquiries concerning the appointment may be made to Dr. R. E. Long (Tel. 091-374-2512).

The post will be of one year's duration starting in Autumn 1989. The appointment will be on Research and Analogous IB (£8,675-£11,680) or IA (£9,865-£15,720) according to qualifications and experience.

Further particulars may be obtained from the **Registrar, Science Laboratories, South Road, Durham, DH1 3LE (Tel. no. 091-374-2264)** to whom applications (three copies), including the names of three referees, should be sent not later than Monday, 17th April, 1989. (8881)A

## UNIVERSITY COLLEGE LONDON

### Department of Biochemistry

### Novel Restriction Enzymes



Technician required for a joint research project with New England Biolabs Inc. to isolate novel restriction enzymes from our extensive culture collection. The position will involve some travel to New England Biolabs laboratories in the USA plus other benefits and can start immediately. Salary £8386 inclusive. Experience of protein purification and/or microbiological techniques would be preferred.

Please telephone or send c.v. plus names of two referees to **Dr. J M Ward, Dept. of Biochemistry, University College London, Gower Street, London WC1E 6BT.** Telephone. 01-387 7050 ext. 2242. Equal Opportunities Employer. (8859)A

## FACULTY POSITIONS MOLECULAR ONCOLOGY AND DEVELOPMENT

Exciting opportunities at levels exist in new programs at Yale University, to be filled over the next 2-3 years. Individuals with research interests in the molecular aspects of oncology or the development of molecular organisms are invited to apply. Qualified candidates, Ph.D. or MD, will have completed at least 2 years of productive postdoctoral research and will be expected to establish independent programs.

Interested applicants should submit a CV, along with a summary of research interests, a list of 3 letters of reference to be sent to: **Dr. Sherman M. Weinman, Search Committee, Yale University School of Medicine, P.O. Box 3333, New Haven, CT 06510.**

An equal opportunity/affirmative action employer. (NW3524)A



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### nature

## BIOCHEMICAL GENETICIST

The Department of Pediatrics, University of Arizona College of Medicine, is seeking a geneticist with primary research emphasis in basic biochemical or molecular genetics to join an active section with one molecular and two clinical geneticists. Qualifications should include the M.D. and/or Ph.D. degree and ABMG board certification or eligibility in clinical biochemical genetics. The position is at the assistant professor level in the tenure track and will be an integral part of a new Mammalian Molecular and Developmental Genetics Unit in The Children's Research Center. Contact: **H. Eugene Hoyme, M.D., Chief, Section of Genetics/Dysmorphology, Department of Pediatrics, University of Arizona College of Medicine, Tucson, AZ 85724. Telephone: (602) 795-5675.** The University of Arizona is an EEO/Affirmative Action Employer. Closing date is 5/15/89 or when position is filled. (NW3528)A

## POSTDOCTORAL POSITION

Available now to investigate mechanisms of regulation of lipoprotein lipase and hepatic lipase gene expression, protein synthesis, and catalytic activities. Cell culture (liver, adipocyte, endothelial), molecular biology, immunochemical (monoclonal antibodies), and recombinant DNA technique approaches are employed. Applicants should have training in biochemistry or molecular biology. Experience with recombinant DNA desirable. Send résumé and names of three references to: **Dr. Andre Bensadoun, Division of Biological Sciences and Nutritional Biochemistry, 321 Savage Hall, Cornell University, Ithaca, NY 14853.** (NW3523)A

## UNIVERSITY OF AUCKLAND New Zealand

DEPARTMENT OF CELLULAR AND  
MOLECULAR BIOLOGY

## POSTDOCTORAL POSITION

To study the effects of cyclic AMP on growth and differentiation of tumour cells, particularly effects on phosphatidylinositol metabolism and possible involvement of oncogenes. Experience in these areas would be advantageous. The position is available for 2 years and includes a return airfare. For further information contact Professor Ray Ralph, Department of Cellular and Molecular Biology, University of Auckland, Private Bag, Auckland, New Zealand. Tel: 64 9 737 999, Fax: 64 9 31 618. (W6004)A

## Staff Scientists/Biochemistry/ Molecular Biology/ Fermentation Technology

CIBA-GEIGY has immediate openings for staff/senior scientists in the Biotechnology Department of its Pharma Research Division. The positions will involve the expression of recombinant proteins in microorganisms, mainly yeast, with emphasis on host strain improvement by physiological/fermentation means.

The qualified individuals will have a PhD in microbiology/biochemistry with a strong background in molecular biology or, alternatively, in biochemical engineering, 2-4 years postdoctoral and/or industrial experience, written and oral communication skills as well as a commitment to team work.

We provide a stimulating professional atmosphere with many opportunities for collaborative projects with small Biotech companies as well as University Institutes. We offer competitive salaries and a comprehensive benefit program. For consideration please forward your CV, list of publications and the name of three referees under ref. "Nature 347" to **Mr. H. Schmid, CIBA-GEIGY Ltd., Personnel Department, P.O. Box, CH-4002 Basle.** (W6008)A

# CIBA-GEIGY

## UNIVERSITY OF READING Lectureships in Microbiology

Applications are invited for two Lectureships in the Department of Microbiology, School of Animal and Microbial Sciences. The preferred areas are Molecular Virology, Bacteriology or Molecular Immunology of Infectious Diseases but applications from candidates with interests in other areas of microbiology will be considered. The appointees will be expected to initiate independent research programmes through obtaining external funding and to contribute to the teaching and administrative commitments of the Department and the School. The Microbiology Department, will shortly move into new purpose built laboratories which will have generous space and excellent facilities and research. Salary scale £9,260 to £14,500 p.a. (Grade A) — under review — plus USS benefits. For particulars and application forms (2 copies) are available from Personnel Office, University of Reading, Whiteknights, PO Box 217, Reading, RG6 2AH, telephone 0734-318751. Candidates from overseas need only send a CV and the names of two academic referees. More information about these attractive posts may be obtained from **Professor J.W. Almond, Department of Microbiology, tel: 0734-318901.** Please quote Ref. AC. 8915. Closing date 24 April. (8838)A

## ST. MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, technology and Medicine)  
(University of London)  
Norfolk Place, London W2 1PG

## WOLFSON POST-DOCTORAL RESEARCH FELLOW

required to join group in the Academic Department of Medicine at St. Mary's Hospital Medical School (Head of Department: Professor H.C. Thomas) to work on the molecular biology of delta hepatitis virus. Previous experience in gene cloning and general molecular biology techniques will be an advantage. Post funded by the Wolfson Foundation for a period of three years with starting salary of £13,330 pa inclusive of London Allowance. Further enquiries to Dr. J. Monjardino (01-725-6381/1606).

Applications in form of a full C.V. with names and addresses of 2 referees should be sent to Personnel Department at above address. Please quote: Ref. 591. (8876)A

# UNIVERSITY of GUELPH

## DEPARTMENT OF VETERINARY MICROBIOLOGY AND IMMUNOLOGY

### Applications are invited for a tenure track position as **ASSISTANT PROFESSOR**

in veterinary immunology. Responsibilities include supervision of the Clinical Immunology Laboratory of the Veterinary Teaching Hospital, research, and teaching. Research opportunities exist in the fields of immunological diseases and diagnostics with the possibility of collaboration and consultation in production and application of conventional and genetically engineered monoclonal antibodies.

Applicants should have a Ph.D. and preferably a DVM degree with experience in laboratory diagnostics and in monoclonal antibody production. A central interest in diagnostic immunology including both applications and development, is essential.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

Please address enquiries to **Dr. Bruce Wilkie, Chairperson, Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.** Applications will be accepted until May 31, 1989 or until the position is filled. Subject to final budgetary approval.

*The University of Guelph is committed to Employment Equity.*  
(NW3519)A

## UNIVERSITY OF NEWCASTLE UPON TYNE THE MEDICAL SCHOOL

### **BAYER LECTURESHIP IN PHARMACOGENETICS**

Applications are invited for the post of Lecturer in Pharmacogenetics, tenable with immediate effect and for an initial period of five years. The postholder will work with Professor J R Idle in the Basic Pharmacology Section of the Department of Pharmacological Sciences and will be expected to play a leading role in the local development of human pharmacogenetics. Candidates should be non-medically qualified, hold a higher degree and have established themselves in the fields of pharmacology, toxicology, drug metabolism or human genetics. Research facilities comprise newly refurbished laboratories provided with gas chromatography, molecular biology and cell culture equipment. The Department is involved in both undergraduate and postgraduate teaching and the successful candidate would be expected to contribute to a broad range of teaching activities.

Salary will be at an appropriate point on the Lecturers' scale A £9,260-£14,500 p.a. or exceptionally on the Lecturers' scale B £15,105-£19,310 p.a. according to experience and qualifications.

Potential applicants are welcome to discuss the post with Professor J R Idle (091-222-7675). Further particulars may be obtained from the **Senior Assistant Registrar, Establishments, The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU** with whom applications (three copies) together with the names and addresses of three referees should be lodged not later than 5th May 1989.

(8877)A

## **RESEARCH ASSISTANT**

Applications are invited for a Research Assistant to work on a project utilising molecular techniques to investigate the mechanisms underlying numerical and structural abnormalities of chromosome 18 in man. The post is funded for a period of three years by Action Research for the Crippled Child and is suitable for

1) a Post Graduate with a first or second class honours degree. (It may be possible for such an applicant to register for a higher degree) or

2) an Individual with a Higher Degree.

Experience in Molecular Genetics is Essential. Salary £8,548-£11,073. Enquiries to **Dr Patricia A. Jacobs or Dr John Harvey at the WESSEX REGIONAL CYTOGENETICS UNIT. Telephone 0722 336212 extension 669.** Application forms from the **Personnel Department, Odstock Hospital, Salisbury, Wilts SP2 8BJ.**

Closing date: 20th April, 1989

(8867)A

## INTERNATIONAL CENTRE FOR MEDICAL RESEARCH (CIRMF) FRANCEVILLE, GABON

### **Reproductive Physiologist/ Primatologist**

A post-doctoral scientist is required to assist in developing a research programme on the hormonal regulation of reproductive behaviour and fertility in non-human primates. The position is available on a two year contract (renewable) commencing Autumn 1989. An attractive salary is offered plus relocation, expatriation and education allowances, housing on the CIRMF campus and 60 days paid annual leave.

Applicants should submit a curriculum vitae and names and addresses of 3 referees to: **Dr G. E. Roelants, Deputy Director General, Scientific Director, CIRMF, BP 769, Franceville, Gabon.** Closing date: 31st May 1989.  
(W6005)A

## THE AFRC INSTITUTE OF HORTICULTURAL RESEARCH

Littlehampton, West Sussex  
Scientific Officer

A Scientific Officer is required in the Entomology and Insect Pathology Section to work on the control of mushroom flies using rhabditid nematodes (*Steinernema*, *Heterorhabditis* spp.) The major aim of this work is to develop biological control strategies both for scarid flies and for the larvae of *cecidiomyiid* pests.

Qualifications: First or Upper Second Class honours degree in appropriate subject or equivalent.

Salary: In range £8,574 - £10,994

Further details and application form quoting Ref 29/L from The Personnel Officer, Bradbourne House, East Malling, Maidstone, Kent ME19 6BJ. Closing date 20th April 1989.

The AFRC is an Equal Opportunities Employer. (8869)A

## **POSTDOCTORAL POSITION NEUROPHYSIOLOGY**

Applications are invited for a post-doctoral position concerning projects with ion-selective micro-electrode techniques on cultured astrocytes and neurons. Applicants should have a background in neurophysiology and experience with electrophysiological recording. Salary will be on MRC (Canada) scales. Applications including CV and names of three referees should be sent to:

**Dr. Wolfgang Walz, Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Sask. S7N 0W0, Canada.**  
(NW3520)A

## UNIVERSITY OF ALBERTA FACULTY OF DENTISTRY

### **ORAL MICROBIOLOGY**

Applications are invited for a tenure-track position in the Division of Pathology in the Department of Oral Biology available from July 1989. Applicants must have a dental degree, and graduate training and at least 2 years postdoctoral research experience in oral microbiology and be prepared to teach in oral microbiology and, to more limited extent, in oral pathology. Preference will be given to applicants with a higher degree (PhD or equivalent) and additional teaching experience in anatomy, biochemistry, physiology, pharmacology or clinical discipline. Rank and salary are commensurate with education and experience of an Assistant Professor (\$32,564-46,700) or Associate Professor (\$40,810-58,954). The University of Alberta is committed to the principle of equity in employment. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents. Applications including curriculum vitae and the name of 3 referees should be sent, no later than April 30, 1989 to:

**Dr D.M. Paton, Professor and Chairman, Department of Oral Biology, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.**  
(NW3522)A

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selection of jobs  
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## UNIVERSITY OF HONG KONG

### Lecturer in Biochemistry

Applications are invited for a Lectureship in the Department of Biochemistry. The appointee will participate in the teaching of students in the Faculties of Medicine, Science and Dentistry. Present research activities in the Department include molecular biology, membrane, developmental and neurobiochemistry, enzymology, immunology and haematology. Consideration will be given to applicants from ANY field of research. Those who are interested in contributing to the teaching of human genetics of lipid biochemistry are encouraged to apply. A higher degree is normally expected and some teaching experience is desirable. Applicants with medical or dental qualifications may have an advantage.

Annual salary (superannuable) is on an 11-point scale: HK\$206,040-44,400 (approx. 15,260-25,510 sterling equivalent as at March 3, 1989). Starting salary will depend on qualifications and experience. A medically qualified appointee who has just completed his pre-registration year will be appointed as Lecturer at the minimum point of the scale. At current rates, salaries tax will not exceed 15.5% of gross income. Children's education allowances, leave, and medical benefits are provided; housing or tenancy allowances are also provided in most cases at a charge of 7.5% of salary.

Further particulars and application forms may be obtained from Appointments (36160), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, U.K., or from the Appointments Unit, Registry, University of Hong Kong, Hong Kong. Closing Date: 9 June 1989. (W6011)A

## UNIVERSITY OF OTAGO Dunedin, New Zealand LECTURER IN BIOORGANIC CHEMISTRY

### Department of Chemistry

Applications are invited from women and men for a Lectureship in the Department of Chemistry. The appointment, part of a programme to attract younger members of staff, is seen to be important in developing a significant chemical presence in biological science in this University.

The main criterion is distinction in research but the successful candidate will be expected to contribute to the undergraduate teaching programme in the Department. Candidates are expected to have a sound background in organic chemistry which will enable them to teach at all levels but the research experience in biological organic chemistry will be interpreted in the broadest sense.

The appointment will be for three years in the first instance and salary will be within the lecturers scale NZ\$35,000-NZ\$42,500 per annum depending on qualifications and experience.

Enquiries may be made to Professor B.H. Robinson, Department of Chemistry, FAX NZ(024) 741-607, by telephoning NZ(024) 797-907 or E-Mail CHEMMAIL@Otago.AC.NZ.

Further particulars concerning the post are available from the Registrar, Mr D.W. Girvan, P.O. Box 56, Dunedin, New Zealand or from Appointments (36168), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, U.K.

Applications quoting reference A89/11 close 22 May 1989.

Equal opportunity in employment is University policy (W6015)A

## DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

### MRC IMMUNOCHEMISTRY UNIT GRADE 4 TECHNICIAN (FULL-TIME or PART-TIME)

BT/291

Candidates are invited to apply for a Research Technician post in this Unit to work on proteins of the immune system. Duties will include assisting with experiments involving recombinant DNA techniques, protein and peptide characterisation, the maintenance of mammalian cell lines as well as the general organisation of the laboratory.

Applicants are expected to have at least ONC/OTEC qualification and have a minimum of three year's relevant experience in a biochemical laboratory.

Salary on the scale £7,505-£8,685 depending upon age and experience. The appointment is for 5 years in the first instance with the possibility of renewal.

Applications including a curriculum vitae and the names and addresses of two referees should be sent to: **The Administrator.**

An Equal Opportunity Employer.

(8878)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD, OX1 3QU.

## UNIVERSITY OF MASSACHUSETTS — AMHERST DEPARTMENT OF CHEMICAL ENGINEERING

invites applications for

### FACULTY POSITIONS

available at all levels. Area of specialization is open, but we are particularly interested in individuals with a strong background in nonlinear dynamics and computation, willing to interact with a wide spectrum of research groups within the department. For Full Professor the department seeks an individual who is an outstanding educator and scholar, and who can provide innovative and dynamic leadership in chemical engineering research and education. Qualifications for the Assistant or Associate Professorship include a Ph.D. in Chemical Engineering, a commitment to graduate and undergraduate teaching, and potential for developing a strong externally sponsored research program. Salary commensurate with qualifications and experience. Applications are accepted up to May 20, 1989.

Please send a resume, brief statement of teaching and research interests, and names of three references to: **Chairman, Search Committee, Department of Chemical Engineering, University of Massachusetts, Amherst, MA 01003.**

The University of Massachusetts is an Affirmative Action/Equal Opportunity Employer. (NW3512)A

## PUBLIC HEALTH LABORATORY SERVICE BOARD

### PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH

### DIVISION OF PATHOLOGY DIAGNOSTICS GROUP

### BASIC/SENIOR GRADE MICROBIOLOGISTS

Graduate or postgraduate microbiologists are required to join the Diagnostics Group.

The work will involve the development of laboratory diagnosis of Arboviral and Bacterial (including Rickettsial) diseases. Candidates should be familiar with current developments in the detection of antigens and antibody applied to the diagnosis of acute

infectious disease.

The period is initially for 3 years and has vacancies for 2 Basic Grade and 2 Senior Grade Microbiologists.

Further information may be obtained from Dr G. Lloyd or Dr D. Rutter, tel: (0980) 610391.

Salary scale: BGM £7378-£11073  
SGM £11778-£15394

NHS terms and conditions will apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Tel: (0980) 610391, to whom completed forms should be returned by 14th April 1989 quoting Post No. 0322/0325. (8863)A

## UNIVERSITY OF CAMBRIDGE DEPARTMENT OF MEDICINE

### Research Technician

Applications are invited for a research technician post in a University funded appointment to study autoimmune responses in human renal disease. The work will involve cell culture, radioimmunoassay and high performance liquid chromatography techniques. Salary in range £7,700-£10,000 per annum depending on qualifications and experience.

### Post doctoral Research Officer

Applications are invited from appropriately qualified scientists to study humoral aspects of autoimmune renal disease in man. Applicants should have experience in molecular biology or immunology. Appointment is Wellcome funded and for two years in the first instance. Starting salary up to £12,000 per annum according to age and experience.

Further details can be obtained from **Dr C.M. Lockwood, School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ (Tel. 0223 336744)** to whom applications should be sent, together with a CV and the names and addresses of two referees. (8875)A



## LEICESTER UNIVERSITY DEPARTMENT OF PHYSICS

### X-ray Astronomy Group

#### DEPUTY MANAGER FOR THE ROSAT WIDE FIELD CAMERA QUICK-LOOK FACILITY

The Wide Field Camera (WFC) is an XUV telescope built by a consortium of five U.K. research groups which will form part of the German/U.K./U.S. X-ray astronomy satellite ROSAT, due for launch in February 1990. In order to monitor the functioning of the WFC and the quality of scientific data returned, a "Quick-Look Facility" (QLF) will be established at the Max Planck Institut fuer Extraterrestrische Physik near Munich. A deputy to the QLF Manager is required to oversee the day-to-day operation of the computer and the execution of procedures to process and analyse data received from the satellite. After an initial period of training in the U.K. the successful candidate will be transferred to Munich about 6 months prior to the launch of ROSAT.

Candidates should have a Ph.D. or equivalent experience, in Physics, Astronomy or a related field, and must have experience in software development and the operation of computers running DEC-VAX/VMS. Experience of data analysis in the field of satellite astronomy and an interest in X-ray astronomy would be advantageous. The post is available immediately and the contract will be for a period of 12 months, though it is expected that funding for the post will be continued for the duration of the ROSAT mission of at least 2 years. Starting salary will be in the range £9,865 to £12,760, on a scale up to £15,720, depending on qualifications and experience. Foreign service allowances will be paid for relocation to Munich.

Applications, which should include a C.V. and names and addresses of two referees, should be sent to **Dr. J.P. Pye, Department of Physics, The University, Leicester LE1 7RH**, by 20th April 1989. Further details are also available from Dr. Pye. (8865)A

**GBF Gesellschaft für Biotechnologische Forschung mbH, Braunschweig, FR Germany** — a government supported Research Institute of Biotechnology — offers two

### Postdoctoral Positions

for two years available immediately to support running research programs in the Department of Genetics on "factors affecting bone cells":

1. Purification, characterization and cloning of a peptide hormone receptor. Experience on assaying receptor-ligand-interaction, on protein purification will be required; familiarity with recombinant DNA-technology is desirable (ref. no. 37/89).
2. Histological analyses with immunotechniques and in-situ hybridisation of bone and various cells that metabolize bone, measurement of bone growth in conjunction with selected growth factors (ref. no. 38/89).

Applications should be addressed to the **Personnel Dept., GBF, Mascheroder Weg 1, D-3300 Braunschweig, FRG**. Please include a complete c.v., list of publications, details of research activities to date and the names and addresses of two referees. Informal enquiries can be made to Dr. H. Mayer (Tel. 531/6181-213). (W6010)A

## UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS

### Division of Biochemistry

Applications are invited for a

### Lectureship in Biochemistry

The successful applicant will be based on the St Thomas's Hospital site and should be prepared to teach also on the Guy's Hospital site.

One of the major interests in the Division of Biochemistry is the Neurosciences but this should not discourage applicants with other backgrounds. Intending applicants should have some post-doctoral experience in Biochemistry or related subjects.

The salary will be on the non-clinical lecturer scale (Grade A) £9,260-£14,500 plus London Weighting Allowance of £1,650, though consideration may be given to exceptional candidates above this range. The appointment is expected to begin on 1 October 1989. Further information can be obtained from Professor H S Bachelard, Division of Biochemistry on 01-928 3619.

Applications with full Curriculum Vitae (six copies), and the names and addresses of two referees should be sent to the **Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** quoting reference STH/BIO/319 by 1 June 1989. (8858)A

## UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts:

ASSOCIATE  
PROFESSORSHIPS/  
SENIOR LECTURESHIPS/  
LECTURESHIPS

DEPARTMENT OF  
BIOLOGICAL SCIENCES

### POST A RANGELAND ECOLOGIST

The Ecologist should have experience in tropical or subtropical rangelands and will be expected to contribute to the MSc Programme in Tropical Resource Ecology. The successful candidate should possess a PhD in the appropriate discipline or equivalent research experience.

### POST B BIOMATHEMATICIAN

A Biomathematician is required: (1) to advise and assist academic staff and postgraduate students in the Department of Biological Sciences with the design analysis and interpretation of research projects as well as the mathematical modelling of Biological phenomena and, (2) to teach courses on basic statistical procedures and computer usage to Biology students at postgraduate and undergraduate levels. Experience in the mathematical analysis and modelling of natural phenomena at all levels including physiology, population and the ecological aspects will be an advantage. A PhD or equivalent experience would be the normal qualifications.

### POST C ENTOMOLOGIST

An experienced Entomologist is required. The lecturer should be capable of contributing to the planning and running of an MSc Programme incorporating Taxonomy, pesticides and other applications, Biological control, Physiology and Biology of crop pests and will also need to teach undergraduate courses in Entomology. A PhD or equivalent research experience would be the normal minimum qualification.

### POST D BOTANIST

A Botanist with wide interests and training is sought. The successful candidate will lecture to first year students in the Plant Kingdom Course and will be expected to

contribute to second and third year teaching and to postgraduate supervision. A PhD or go Honours degree plus equivalent research experience would be the normal minimum qualification.

### POST E ANIMAL ECOLOGIST

The successful applicant will be responsible for undergraduate courses, in addition to teaching and supervisory contributions, an MSc Programme in Tropical Ecology. Ideally, candidates should have a proven record in the supervision of postgraduate students preferably in large mammal ecology, although other areas may be considered. A PhD or equivalent research experience would be the normal minimum qualification.

### DEPARTMENT OF CHEMISTRY (PHYSICAL CHEMISTRY)

Candidates should have a relevant PhD degree and lecturing research experience. Preference may be given to candidates oriented towards reaction kinetics and thermodynamics and their applications.

**SALARY SCALE:** Non-Medical Lecturer: Z\$19,860-Z\$28,170; Senior Lecturer: Z\$29,170-Z\$31,572; Associate Professor: Z\$32,532-Z\$33,624. Appointment on the above scales will be according to qualification and experience.

### CONDITIONS OF SERVICE

Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended.

Applications should quote a reference number of the advertisement (ASA/7/02/89) and submit six copies of applications giving full personal particulars which should include full name, place and date of birth, qualification, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees, to the Deputy Registrar (Administration) University of Zimbabwe, PO Box MP167, Mount Pleasant, Harare, Zimbabwe (Telex: 4-152 ZW).

Applicants from outside Zimbabwe should also send a copy to Appointments, Association of Commonwealth Universities, Gordon Square, London WC0E 0PF, U.K.

Closing date for the receipt of applications is 14 April 1989.

(W6013)A

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## RESEARCH ENGINEERS Caltech Submillimeterwave Observatory

The California Institute of Technology in Pasadena, CA operates a submillimeterwave observatory at the Mauna Kea site in Hawaii. Receivers, spectrometers, and electronic and computer support systems are being constructed to allow operation in both radiometric and spectroscopic modes, throughout the frequency range 200-1,000 GHz. Positions are available for engineers, physicists or astronomers with experience in any of the following areas: radio electronics, radio astronomy techniques, millimeter or submillimeter receivers, liquid helium temperature cryogenics, bolometers and superconducting tunnel junction techniques. Senior and junior positions are available at Caltech in Pasadena. Some additional job opportunities associated with the Caltech observatory are available in Hawaii.

Applicants should have at least a B.S. degree in engineering, physics or astronomy.

Applications should be sent to Manager of Employment, California Institute of Technology 101-6, Pasadena, CA 91125, and should include a summary of education and relevant job experience.

Caltech is an Affirmative Action/Equal Opportunity employer. Women and minorities are encouraged to apply (NW3527)A

## DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

### GENETICS LABORATORY POSTDOCTORAL RESEARCH ASSISTANT — Grade 1A BR/290

A Postdoctoral Research Assistant is required to join a team comparing the applications of pulsed field electrophoresis and the polymerase chain reaction with standard methods of carrier diagnosis in Duchenne Muscular Dystrophy.

Position available for one year with potential for renewal. Based at the Churchill Hospital, in close collaboration with the Genetics Laboratory in the Department of Biochemistry.

Salary on the scale £9,865-£15,720 per annum.

Applications, including the names and addresses of two referees, should be sent, as soon as possible to: **The Administrator.**

*An Equal Opportunity Employer.*

(8879)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD, OX1 3QU.

### THE UNIVERSITY OF MANCHESTER RESEARCH ASSISTANT/ASSOCIATE IN THE DEPARTMENT OF MEDICINE

Applications are invited for this post in the University Hospital of South Manchester, to examine cellular interactions in human melanoma. The appointee will have experience of cell culture and protein chemistry techniques. This is a two year appointment supported by the R.L. Gardner Bequest Fund at Withington Hospital. Salary in range £8,675-£12,150 p.a. according to qualifications and experience. Applications, with the names of two referees should be submitted to

**Dr. D. Woolley, University Hospital of South Manchester, West Didsbury, Manchester M20 8LR,** as soon as possible.

*The University is an equal opportunities employer.* (8851)A

Delta Biotechnology Limited, a subsidiary of Bass Plc, specialises in the manufacture of human healthcare products. The Company is located in central Nottingham where it is now building a large pilot plant, designed for the production of rDNA products in compliance with Good Manufacturing Practice.

We are now commencing a phased recruitment schedule for this plant, the first phase of which is to attract the key people to form the plant managerial team and to become involved from the start of equipment installation through to successful operation.

These positions will require people with an excellent track record within the pharmaceutical/biotechnology industry and a high level of commitment to the successful operation of this plant.

## Senior Fermentation Supervisor

This position requires a good academic background (Life Sciences/Biochemical Engineering) in addition to at least 5 years industrial experience within the area of fermentation and cell separation at pilot plant/production scale. The ability to communicate with all levels of staff is essential and the candidate must be able to demonstrate good supervisory skills. Experience of working in compliance with GMP guidelines is highly desirable.

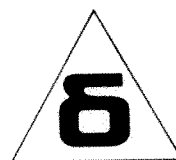
## Senior Downstream Processing Supervisor

This position requires a good academic background (Life Sciences/Biochemical Engineering) in addition to at least 5 years industrial experience within the area of downstream processing at pilot plant/production scale. Experience of working in compliance with GMP guidelines and the ability to communicate with all levels of staff is essential. The candidate must be able to demonstrate good supervisory skills and experience of clean room/sterile room operations would be an advantage.

Attractive remuneration packages commensurate with age and experience will be offered with regard to the above positions.

Please write with full c.v. to:-

**Peter Lees, Delta Biotechnology Ltd.,  
Castle Court, 59 Castle Boulevard,  
Nottingham NG7 1FD.**



**DELTA**  
BIOTECHNOLOGY  
LIMITED

(8884)A

## GREENADDER INDUSTRIAL

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(8495)A

## ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

### Anatomical Society Research Studentships

Applications are invited from Departments of Anatomical Sciences in the UK and Ireland for Research Studentships tenable in the Anatomical Sciences for a period up to three years. Students nominated by Departments must be graduates of British or Irish Universities and will be expected to register for a higher degree. The stipend will be commensurate with basic Research Council rates.

### Anatomical Society Senior Visiting Fellowship

Applications are invited from Overseas Scientists for one Fellowship tenable for a period of less than a year in a Department of Anatomical Sciences in the UK or Ireland. Applicants should be of post-doctoral or comparable status and must have arranged sponsorship in the Department in which they intend to work. Some assistance towards travel and subsistence will be provided.

Further particulars should be obtained from the Acting Secretary, Dr Ian Whitmore, Department of Cell and Structural Biology, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, to whom applications should be sent by 28 April 1989.

(8606)A

## PUBLIC HEALTH LABORATORY SERVICE BOARD PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH DIVISION OF BIOLOGICS PROTEIN CHEMISTRY SECTION

Biochemist/Immunologist (Senior Grade Microbiologist) — Post No. 0550

A biochemist is required to join a research group involved in the study of bacterial protein toxins. The successful applicant will be responsible for developing immunoassays for several protein toxins with an emphasis on producing novel, rapid assay methods. Applicants must have drive, be able to work independently and should have at least four years postgraduate experience preferably with a PhD in a relevant field. Experience in hydridoma technology and immunoassay techniques would be an advantage although not essential.

Salary will be in the range £11,778-£15,394, depending on experience.

The position will be funded initially for a period of three years.

NHS terms and conditions will apply.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Tel: (0980) 610391, to whom completed forms should be returned by 14th April 1989 quoting Post No. 0550 (8862)A

## Imperial College of Science, Technology and Medicine. (University of London) BIOCHEMISTRY DEPARTMENT RESEARCH ASSISTANT

A graduate research assistant is required to join a small group engaged in producing a physical map of the short arm of human chromosome 11. The project involves cosmid cloning, DNA fingerprinting and data analysis by computer. Some experience in DNA manipulation is desirable, but not essential.

Salary on RAIB scale from £8675-£11680 depending on age and experience plus London Allowance £1650. This position is available immediately.

Applications with CV and names of 2 referees and informal enquiries to Dr. P.F.R. Little, Biochemistry Dept., Imperial College of Science, Technology and Medicine, Imperial College Road, London, SW7 2AZ. Tel: 01-589-511 EXT. 4164. Ref: BIO/AD/24 (8866)A

## IMMUNOLOGIST / VIROLOGIST

Laboratory of Infectious Diseases  
National Institute of Allergy and Infectious Diseases  
National Institutes of Health

Position available immediately for qualified immunologist or virologist to work in the Respiratory Viruses Section, NIAID, NIH. Work will involve a detailed characterization of the cellular immune response to respiratory syncytial viruses (RSV) proteins and an investigation of the immunological mechanisms underlying the enhanced disease seen in recipients of inactivated virus vaccine following natural infection with RSV. A Ph.D. in immunology, virology or molecular biology is required.

Salary range: \$24,000 - \$27,000

Qualified and interested candidates should send curriculum vitae, Application for Federal Employment (SF-171) and names and addresses of three (3) references to:

Brian R. Murphy, M.D.  
Head, Respiratory Viruses Section  
NIAID, NIH  
Bldg. 7, Room 106  
9000 Rockville Pike  
Bethesda, MD 20892 (NW3517)A

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**SCIENCE IN EUROPE**  
27th April 1989

**nature**

**This special feature issue of *Nature* will focus on the present pattern of science in Europe.**

Vacancies, fellowships, symposia, conferences, lectures, workshops, announcements will all benefit from the extra interest generated by this truly "European" issue.

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London — Julie Skeet: Tel: 01-836 6633 Fax: 01-240 2408	Munich — Sabine Fürst: Tel: (089) 26 50 31 Fax: (089) 26 93 24	Paris — Clare Newell: Tel: (1) 40 53 03 39
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## POSTDOCTORAL POSITIONS

### EUKARYOTIC MOLECULAR GENETICS

Available immediately for study of the genetics of the opportunistic AIDS pathogen, *Pneumocystis carinii*. Experience in molecular genetics, biochemistry or molecular pathogenesis required. Send c.v. and three letters of reference to: Dr. G. A. Buck, Box 678 MCV Station, Va Commonwealth Univ., Richmond, Va. 23298.

## POSTDOCTORAL RESEARCH ASSOCIATE

to develop nonconventional systems for crop improvement. Two-year appointment at \$22,000 per year, available immediately. Funded by the Christine Lee Shepard Memorial Fund, established to promote innovative research for plant improvement. Required: Ph.D.; training in recombinant DNA and/or plant tissue culture. Send curriculum vitae, including coursework transcripts, a statement of research interests, and have three letters of reference sent by 15 April 1989 to: Dr. Frank White, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506. Telephone: 913-532-6176. *Equal Opportunity/Affirmative Action Employer.*

## MOLECULAR IMMUNOLOGIST BRANDEIS UNIVERSITY

The Rosenstiel Research Center and the Department of Biology, seek a tenure-track Assistant Professor in the area of the molecular biology of the immune system. Candidates should have a strong record of research accomplishments in this area. Exceptional individuals at a more senior level will also be considered. Please submit a curriculum vitae, including a description of research goals, and the names of three individuals who can supply letters of recommendation to: Molecular Immunology Search Committee, Rosenstiel Center, Brandeis University, Post Office Box 9110, Waltham, MA 02254-9110, but June 15, 1989. *Brandeis University is an Equal Opportunity/Affirmative Action Employer.*

# Toxicology

## Associate Director, Clinical Toxicology & Safety Evaluation

Smith Kline & French, a leader in Pharmaceutical research, is consolidating and expanding its Safety Assessment Departments. The Department of Toxicology is seeking an experienced senior scientist for the position of Associate Director. This individual will provide scientific leadership and management for two expanding groups of scientists and technicians engaged in clinical and pharmacological evaluations of novel therapeutic agents in animals.

The successful candidate will participate with multi-disciplinary teams in drug discovery and development and will have responsibility for designing and conducting studies to define the toxicologic profile of novel therapeutic agents. The incumbent also would manage a group responsible for evaluating the pharmacologic and physiologic effects of therapeutic candidates on major organ system function and relating those effects to the overall determination of safety.

Candidates with a DVM or MD and a Ph.D. in pharmacology, physiology or toxicology would be ideally qualified for this position. A proven record of accomplishment in experimental and/or clinical research in one of the above areas is required. The successful candidate may be currently employed in an academic or research institution but experience in the Pharmaceutical industry would be advantageous. Board certification in internal medicine would be desirable.

Based in a modern research facility in suburban Philadelphia near Valley Forge, PA, SK&F provides excellent opportunities for innovative, interdisciplinary research in a stimulating and challenging scientific environment. Attendance at national and international scientific meetings is supported and presentation and publication of scientific work is strongly encouraged. We offer an attractive compensation/benefits and relocation package. For confidential consideration, please forward your C.V. and names of three references to Elizabeth McKendry, L0051, Associate Employment Administrator, Smith Kline & French Laboratories, Research & Development, P.O. 1539, King of Prussia, PA 19406-0939. *Equal Opportunity Employer, M/F/H/V.*

**SK&F**  
A SmithKline Beckman Company

## HANS SELYE SYMPOSIA ON NEUROENDOCRINOLOGY AND STRESS: NEUROENDOCRINOLOGY OF GASTROINTESTINAL ULCERATION

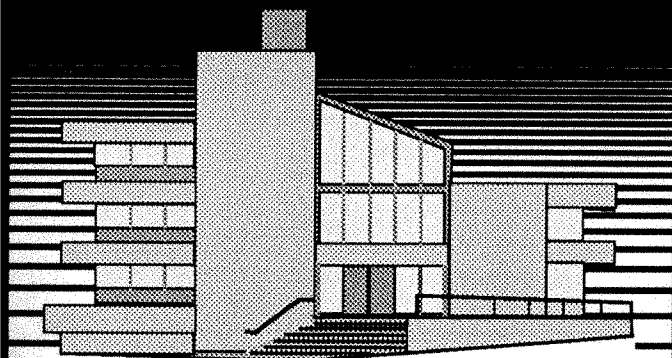
September 13-15, 1989

Estérel (near Montreal), Quebec, Canada

The "Hans Selye Symposia on Neuroendocrinology and Stress" are held every 2-3 years and are devoted to specific topics in fields related to neuroendocrinology and stress. The next meeting will be devoted to the neuroendocrine aspects of gastrointestinal (GI) ulceration. The *format* of the meeting will be that of mini-symposia where following 2-3 invited lecturers, brief presentations will be scheduled selected from submitted abstracts. The *topics* to be covered are the neuroendocrine control of gastroduodenal secretion, GI motility, GI microcirculation, gastric and duodenal ulceration, ulcerative colitis, as well as central vs. peripheral neuroendocrine factors in ulcer disease, growth factors and immunology as related to neuroendocrinologic aspects of GI ulceration. *Deadline* for submission of abstracts is May 1, 1989 and for registration to participate without abstract presentation is August 15, 1989. The abstracts will be published in Digestive Diseases and Sciences. Registration material and abstract forms can be obtained from Joyce M. Fried, Brain Research Institute, University of California, Center for the Health Sciences, Los Angeles, CA 90024-1761. Telephone: (213) 825-5061, FAX (213) 206-5855.

The Standing Committee of the Hans Selye Foundation: Mark Cantin (Montreal), Louise Drevet-Selye (Montreal), Sandor Szabo, coordinating chairperson for this meeting (Boston) and Yvette Taché (Los Angeles).

## THE BIOMEDICAL RESEARCH CENTRE



### POST-DOCTORAL AND GRADUATE STUDIES OPPORTUNITIES

The Biomedical Research Centre (BRC) is located on the campus of The University of British Columbia. Research at The BRC focuses on the regulatory mechanisms of cellular growth and differentiation, with a particular emphasis on the roles of cytokines, receptors and post-receptor signal transduction. The facility is equipped for molecular and cellular biology, as well as protein purification, and automated sequencing and chemical synthesis of proteins. Current research areas include: the molecular regulation of steady-state lymphopoiesis and hemopoiesis; the roles of cytokines in autoimmune diseases, infection and tumorigenesis; immunological tolerance and models of human disease using transgenic animals, retroviral gene transfer and homologous recombination; structure-function analysis of the polypeptide and carbohydrate moieties of the lymphokines IL-3 and GM-CSF and their receptors; and the molecular and functional characterisation of cell cycle-regulated protein kinases. The cross-disciplinary exchange of ideas and expertise within its interactive environment makes The BRC ideal for career development.

APPLICANTS SHOULD SEND THEIR CURRICULUM VITAE, A COPY OF RECENT TRANSCRIPTS AND NAMES OF THREE ACADEMIC REFEREES TO:

**The Director,  
The Biomedical Research Centre,  
2222 Health Sciences Mall,  
The University of British Columbia,  
Vancouver, B.C. CANADA V6T 1W5**

### POSTDOCTORAL RESEARCH DRUG DELIVERY SYSTEMS/ DRUG TARGETING

Postdoctoral positions are available in a laboratory studying biological approaches to drug targeting in cancer therapeutics. Delivery systems include liposomes, polypeptides and oligonucleotides. A background in pharmaceuticals or in cancer pharmacology is desirable. Positions are offered for two years with possibility of renewal. Competitive salaries. Please submit a curriculum vitae and names of two references to:

**Prof. R. J. Juliano  
Dept. of Pharmacology  
CB# 7365, Fac. Lab. Off. Bldg. 1106  
School of Medicine  
University of North Carolina at Chapel Hill  
Chapel Hill, NC 227599-7365**

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### POSTDOCTORAL POSITION IN PROTEIN CRYSTALLOGRAPHY

to solve the structures of the GCN4 leucine zipper, yeast nucleoside diphosphokinase or the phage P22 tail spike. Requirements include 2 to 3 years of experience with data collection, computing, phase determination, structure analysis and graphics. Send c.v. and letters of reference to Tom Alber, Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah 84132. *The University of Utah is an equal opportunity/affirmative action employer.*

### TWO POSTDOCTORAL POSITIONS

available 1 July 1989. One position involves the investigation of cytoplasmic factors regulating DNA synthesis in lymphocytes. The second involves mechanisms of tumor cell adhesion to endothelial and parenchymal cells and regulation of this interaction of cytokines. Experience in molecular biology, biochemistry, and/or protein purification is desired; knowledge of immunology would be helpful. Send curriculum vitae, statement of interests, and names and addresses of three references: Dr. Stanley Cohen, Department of Pathology, Hahnemann University, Broad and Vine Streets, M.S. 435, Philadelphia, PA 19102-1192.

### POSTDOCTORAL POSITION Molecular Neurobiology

Available immediately to study the structures and functions of synapse-specific proteins using molecular cloning, protein chemistry and cell biology techniques. Send Curriculum Vitae and names of three references to: Dr. Thomas C. Südhof, Howard Hughes Medical Institute, Department of Molecular Genetics, UT Southwestern Medical Center Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235-9050, USA.

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FELLOWSHIPS(36176) Filamentous Fungal  
Genetics

The successful applicant will join a research programme in the Department of Microbiology and Genetics aimed at developing transformation and gene replacement techniques in the ryegrass endophyte *Acremonium loliae*. The position will be available for up to two years. Applicants should have a PhD in an appropriate discipline with experience in Molecular Genetics or Biochemistry. A background in filamentous fungal genetics would be an advantage. Further information may be obtained from Professor D B Scott (tel (063) 69 099 ext. 7965 or Fax (063) 62 140).

Closing date: 30 April 1989.

(36177) Animal Science  
Department

A postdoctoral position is available in the Department of Animal Science, supervised by Drs H T Blair and S N McCutcheon, to conduct physiological studies of the regulation of seasonal wool growth in Romney sheep. This project, which is part of a collaborative research programme, will investigate the physiological mechanisms by which genetic variation in seasonality of wool growth (particularly the winter decline) is expressed. Studies will be based on the University fleece-weight selection lines which exhibit marked genetic variation in seasonality. Applicants should be experienced in the study of whole body and/or tissue metabolism, particularly as it relates to utilisation of amino acids for wool growth and/or endocrine regulation of wool growth. The Fellowship is tenable for a maximum period of two years.

Closing date: 12 May 1989.

Salary: NZ\$35,000 per annum.

Applications, including a full curriculum vitae and naming three referees, should be sent to Mrs V B Bretherton, Personnel Section, Massey University, Palmerston North, New Zealand by the appropriate closing date. (W6012)E

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## SANDOZ INSTITUTE FOR MEDICAL RESEARCH

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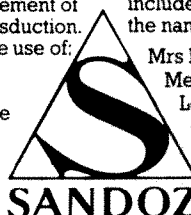
These positions form part of an integrated, multidisciplinary study of the actions of neurotransmitters and chemical mediators on sensory neurones and the involvement of second messengers in sensory transduction. The investigations would involve the use of:

1. whole cell and single channel recording techniques and
2. calcium indicator dyes to measure intracellular calcium in single cells.

Salary will be in the range £14,000 - £17,000, depending on age and experience. Applications, which should include a resumé of research to date, plus the names of two referees should be sent to

Mrs M.-C. Stuart, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN

by Monday 24th April 1989.



(8883)E

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To commemorate the 1969 bicentenary of Captain Cook's first landing in New Zealand on 9 October 1769 the New Zealand Government established an international research fellowship to perpetuate Captain Cook's spirit of scientific enquiry and exploration in New Zealand and the South-West Pacific.

The fellowship is awarded to one person of senior status for two years (extendable to three) to carry out research within New Zealand or the South-West Pacific region in any appropriate field such as the following subjects (taken in their broadest sense) or in any other relevant field:

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**Biology**  
**Geography**  
**Geology**

**Geophysics**  
**History**  
**Medicine**  
**Oceanography**

The successful applicant typically would have at least 5 years post-doctoral experience, have published results of original research and be between 30 and 50 years old. All applicants must have a good working knowledge of English. The successful applicant will be based at a New Zealand university or research institution. The salary will be up to the maximum for an associate professor in New Zealand universities (within the range NZ\$63,800-NZ\$72,000 per annum). A travel grant will be paid equivalent to economy air fare to and from New Zealand for the fellow, spouse and dependent children under 16, plus an allowance for the transport of limited personal effects. Reasonable travel expenses incurred in connection with the research will also be paid.

The successful applicant will be expected to take up the fellowship within six months of appointment in September 1989.

**Applications closing 30 June 1989** are invited for the eighth Captain James Cook Fellowship to be taken up in early 1990. Fellowship brochures and application forms may be obtained from any New Zealand Embassy or High Commission or from the Royal Society of New Zealand, Box 598, Wellington, New Zealand.

(W6009)E



## THE MORRISON MEMORIAL POSTDOCTORAL FELLOWSHIP

The Department of Biochemistry, St. Jude Children's Research Hospital, is enlarging its research efforts in the area of the molecular and cellular biology of signal transduction. As part of this expansion, the Board of Governors has established a memorial fellowship in honor of Dr. Martin Morrison, the founding chairman of the Department. The research programs within the Department are well equipped and offer recent Ph.D. or M.D. graduates the opportunity to participate in energetic, multidisciplinary groups dealing with a range of basic and clinically oriented research. Current research programs within the Department include:

- W.Y. Cheung: Regulatory properties and biological functions of calmodulin;  $\text{Ca}^{2+}$  regulated enzymes.
- J. Cleveland: Growth factor regulation of gene transcription.
- V.A. Fried: The ubiquitin system and post-translational regulatory pathways.
- J.N. Ihle: Mechanisms of hematopoietic stem cell growth regulation, differentiation and transformation.
- S. Jackowski: Regulation of phospholipid metabolism and the production of lipid-derived second messengers.
- C. Rock: Hormone and growth factor stimulated phospholipase C.
- E. Thomas: Antimicrobial and antitumor mechanisms of leukocytes.

Applicants should submit a brief statement of their research interests, curriculum vitae, reprints and names of three references to:

**The Morrison Postdoctoral Fellowship**  
**Department of Biochemistry**  
**St. Jude Children's Research Hospital**  
**332 N. Lauderdale**  
**Memphis, TN 38101**

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 (NW3401)E

## STUDENTSHIPS

### ANIMAL HEALTH TRUST Department of Comparative Physiology Balaton Lodge, Newmarket, Suffolk

Applications are invited for a

### PhD STUDENTSHIP

(£7,000 pa) tenable for 3 to 4 years commencing October 1989. Applicants should have, or expect to obtain, a First or Upper Second Class Honours degree in biochemistry or a related discipline.

The research will be undertaken in a Department actively involved in investigating various aspects of exercise physiology and biochemistry of the racehorse. The specific project will be concerned with factors influencing ascorbic acid status in the exercising horse.

*Application forms can be obtained from*  
**C G Cook, Personnel Officer**  
**Animal Health Trust, P O Box 5**  
**Newmarket, Suffolk CB8 7DW**

*Informal enquiries may be addressed to*  
**Dr D Snow (tel: 0638) 661111**

Closing date for applications: 28 April 1989. (8852)F

## UNIVERSITY OF DUNDEE DEPARTMENT OF ANATOMY & PHYSIOLOGY MRC POSTGRADUATE STUDENTSHIP WITH BURSARY

An earmarked Studentship has been awarded to Professor M J Rennie who directs a group investigating fundamental and clinical aspects of fuel and amino acid metabolism in health and disease. The work is currently supported by MRC, SERC and major charities such as Action Research, British Diabetic Association and The Wellcome Trust by awards of about £150K p.a. Applications are invited from prospective graduates (2i degree expected) in any area of the life sciences, chemistry or physics. The project will involve expression of mRNA for mammalian amino acid transporters in *Xenopus* oocytes with the aim of obtaining information about the molecular mechanisms of amino acid transport and cloning the transporter molecules. A bursary of £250-£500 will be added to the first year stipend, according to class of degree awarded.

Studentships or Research Assistantships may be available in other fields; details may be had on application.

The Department has excellent facilities for research and has recently been awarded £900K for refurbishing additional accommodation. Dundee offers outstanding value for accommodation and access to outdoor recreation, within a friendly community.

Applications (with full c.v. and names and addresses of two referees) as soon as possible to **PROFESSOR M J RENNIE, DEPARTMENT OF ANATOMY & PHYSIOLOGY, THE UNIVERSITY, DUNDEE DD1 4HN (Tel 0382 23181 ext 4572)** who will provide a copy of the Departmental Booklet and information about Tayside. Travel and accommodation expenses will be paid for applicants invited for interview. (8873)F

## DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

### MRC IMMUNOCHEMISTRY UNIT RESEARCH STUDENTSHIP

Applications are invited for a MRC Research Studentship tenable for 3 years, commencing October 1989, to work towards a D.Phil. degree with Dr. S.K.A. Law on the Molecular Biology of the Complement Receptor Type III. The project will focus on the expression of a soluble form of the antigen and the characterization of its higher order structure. It will involve the use of techniques in recombinant DNA and protein chemistry.

Candidates should have, or expect to obtain, a first or upper second class honours degree in one of the biological science disciplines.

Applications, including a complete CV and the names and addresses of two referees, should be sent to the Director, MRC Immunochemistry Unit, by 15th May, 1989. (8880)F

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### ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London) Rowland Hill Street London NW3 2PF

### Ph.D. RESEARCH STUDENTSHIP

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Informal enquiries to **Dr. C. A. Greig, Academic Department of Geriatric Medicine (01-794-0500 x4351)** to whom applications, including a c.v. and names and addresses of two referees should be sent by 24 April 1989. (8843)F

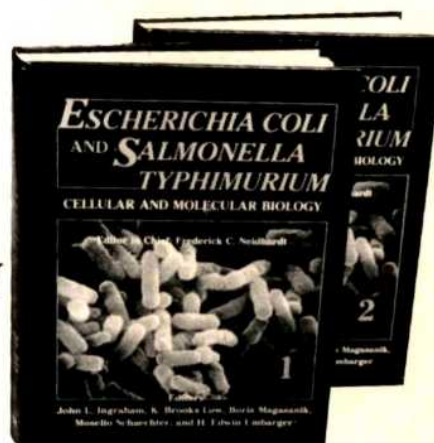
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The two volumes are divided into six parts, with a total of 104 chapters written by about 150 authors who are recognized authorities. The total number of literature citations must be nearly 20,000. Despite these encyclopaedic dimensions and its multi-author make-up, the treatise is remarkably free from the overlap and the imbalance that frequently mars this kind of enterprise. It

is equally pleasing to be able to report that the majority of authors have succeeded in writing what their editors required of them: "thoughtful and narrative reviews" as opposed to mere compilations of data and references. . . . Overall we and other colleagues are very impressed by these books. . . . The editors have obviously put a lot of effort into this enterprise and played an effective disciplinary role in maintaining breadth without overlap, and a uniform style. There is nothing comparable available on the market and everyone working with, or teaching about, *E. coli* and *S. typhimurium* will find these books to be invaluable. Final year undergraduates and postgraduate students will also find them an excellent resource."

B. M. Wilkins and R. H. Pritchard, *Nature*

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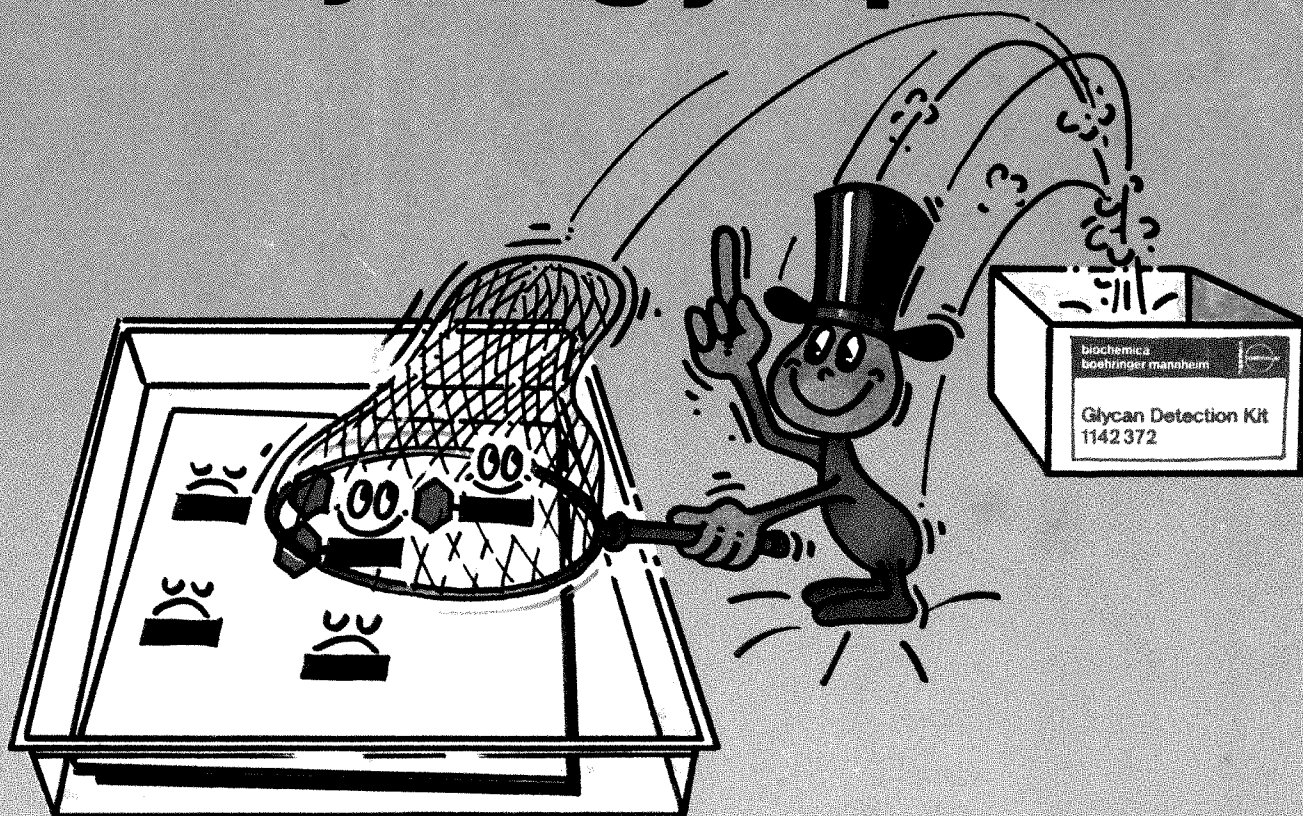


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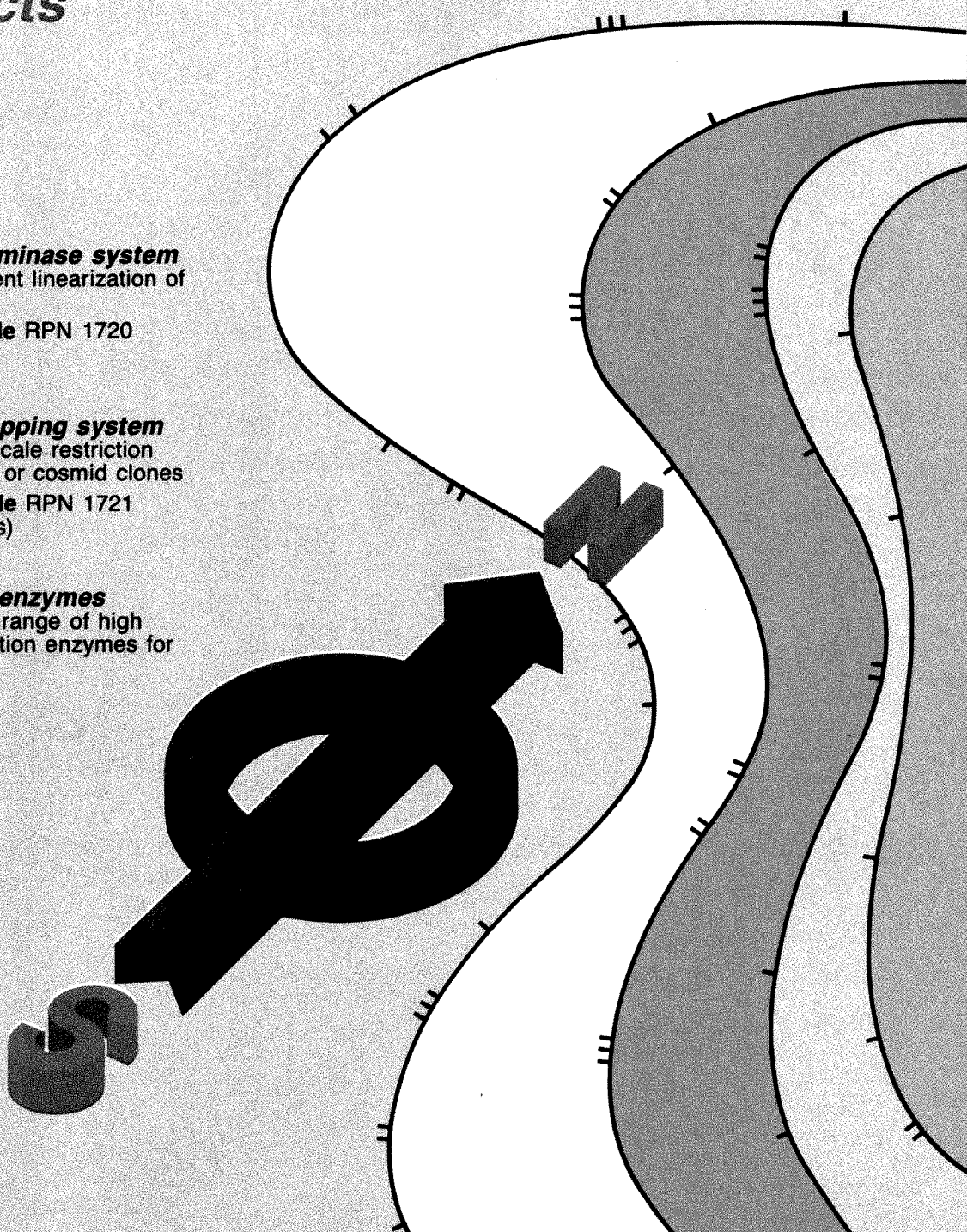
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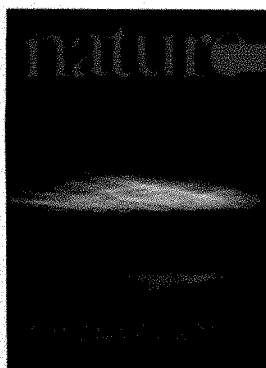


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# nature

6 April 1989

Vol. 338 Issue no. 6215

Noctilucent ice clouds, first reported in 1885, may be a result of the increasing level of atmospheric methane, page 490. The cover photograph was taken at Kustavi in Finland on 3–4 August 1986 by Pekka Parviainen 2 s exposure, 50 mm lens, (f/1.4 on Kodachrome KR 64.)

## THIS WEEK

### Light-weight tweezers

The recently developed 'optical tweezers' use the force exerted by light to manipulate small objects, such as intact cells. Optical tweezers have now been used to investigate the elastic properties of bacterial flagella, page 514.

### Exhaust clean-up

Automotive engineers are working on ways to cut emissions of nitrogen oxides from the internal combustion engine. But in one promising system, the oxygen also emitted during combustion limits efficacy, page 492.

### Electrophoresis gels

Observations of individual DNA molecules during gel electrophoresis by fluorescence microscopy should lead to insights into the basis of this much used, but poorly understood technique. Pages 520 and 461.

### Metals in the making

Deep beneath the East China Sea, hydrothermal vents are in the process of forming the 'Jade deposit', a modern-day analogue of the Kuroko-type sulphide deposits rich in the metals zinc, lead, copper, gold and silver. Page 496.

### Samoaan odyssey

In a new film, Derek Freeman continues his critique of Margaret Mead's anthropological work in Samoa. Adam Kuper looks at on both the film and the wider issues. Commentary, page 453.

### Fractal earthquakes

Theories of chaos, developed in diverse fields, are being brought to bear on studies of the destructive forces of plate tectonics. Page 459.

## Torso a receptor?

The torso gene in *Drosophila* is the archetype of one of three classes of genes that specify the anterior-posterior axis of the embryo during oogenesis. Sequencing of the gene shows that its product is likely to be a transmembrane protein with an intracellular tyrosine kinase domain, page 478.

## Mars bar

On page 487, the effects of meteoritic impacts are proposed as a mechanism that explains the loss of atmosphere on Mars. The model predicts a primordial martian atmospheric pressure of about one bar, or one (Earth) atmosphere. On the same basis Earth's primordial atmosphere would have been about six times today's value. See also News and Views, page 465.

## Educating T-cells

Tests of the ability of isolated epithelial cells to present antigen suggest that their major histocompatibility complex molecules differ from those on other tissues, binding a broad spectrum of T-cell receptors and thus offering a basis for selection of thymocytes biased towards recognition of self-MHC plus antigen. Page 503.

## Muscular dystrophy

Using the polymerase chain reaction, eight different forms of dystrophin have now been detected, with differing tissue distribution. Page 509.

## Ageing challenge

The case for slowing of the ageing process and disease by dietary restriction is presented in a new book reviewed by Alex Comfort, page 469.

## Guide to Authors

Facing page 522.

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Autonomy must be an essential ingredient in the restructuring of British universities ■ Computerization of *Nature* subscriptions

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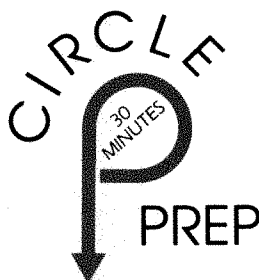
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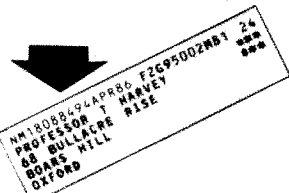
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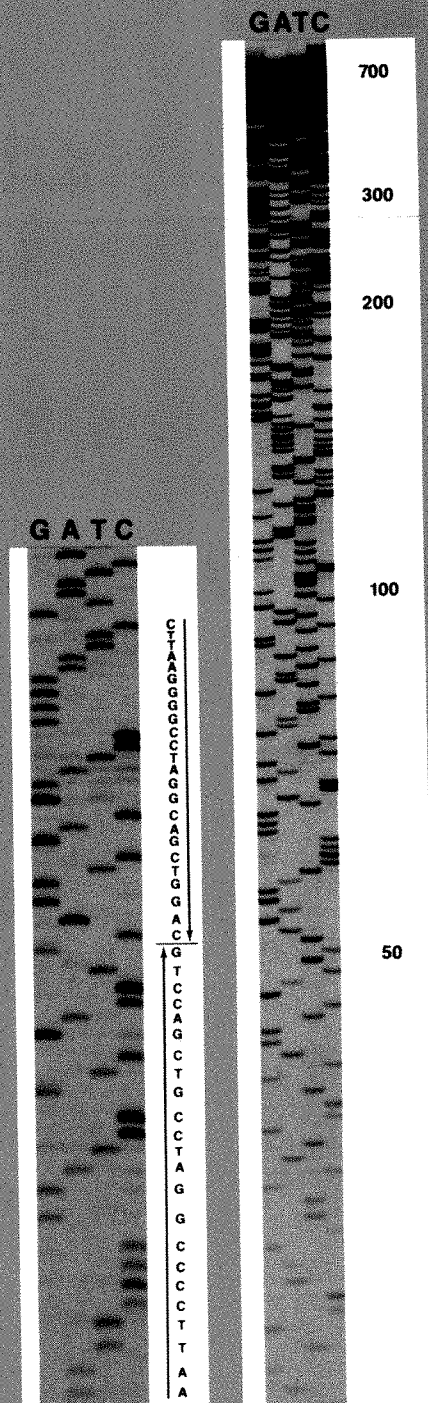
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**Figure 1**

**Figure 2**

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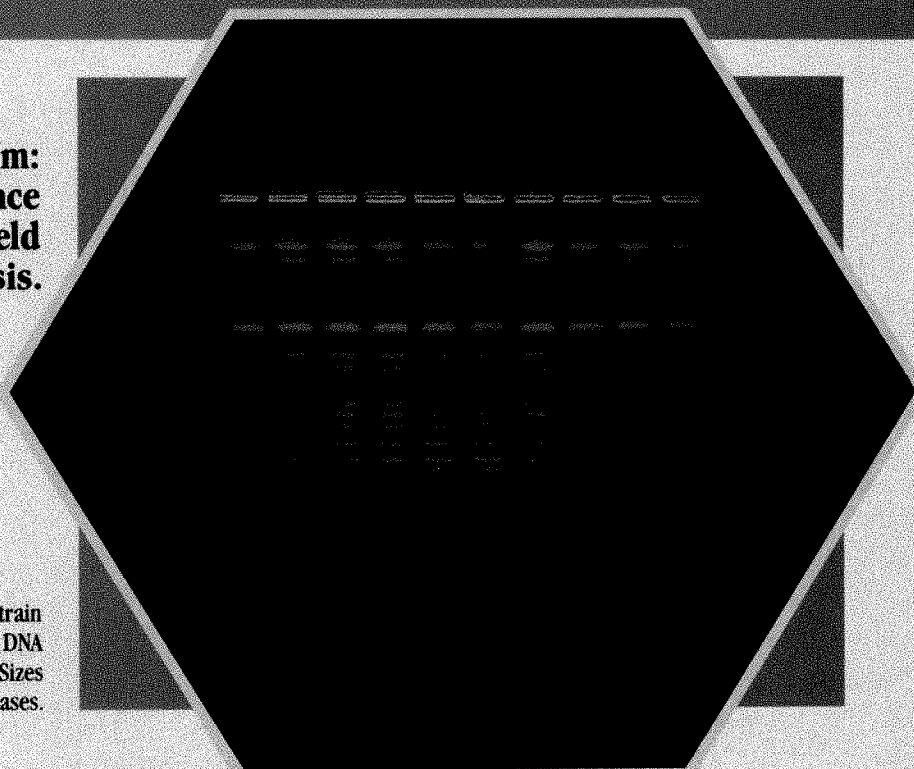
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<sup>1</sup>Chu, G., Vollrath, D. and Davis, R., *Science*, 232, 1582 (1986).

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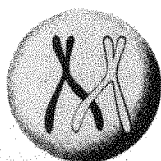
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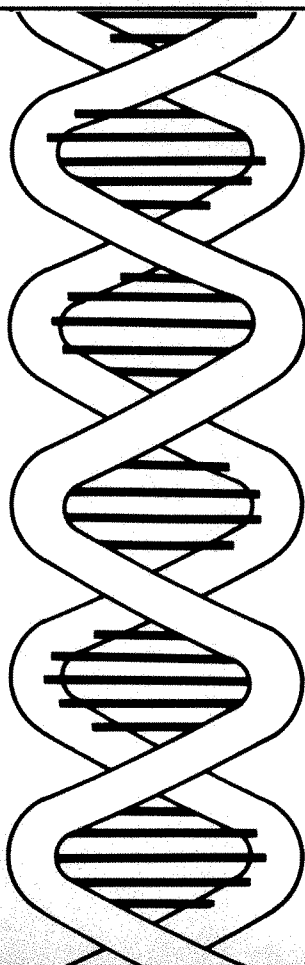
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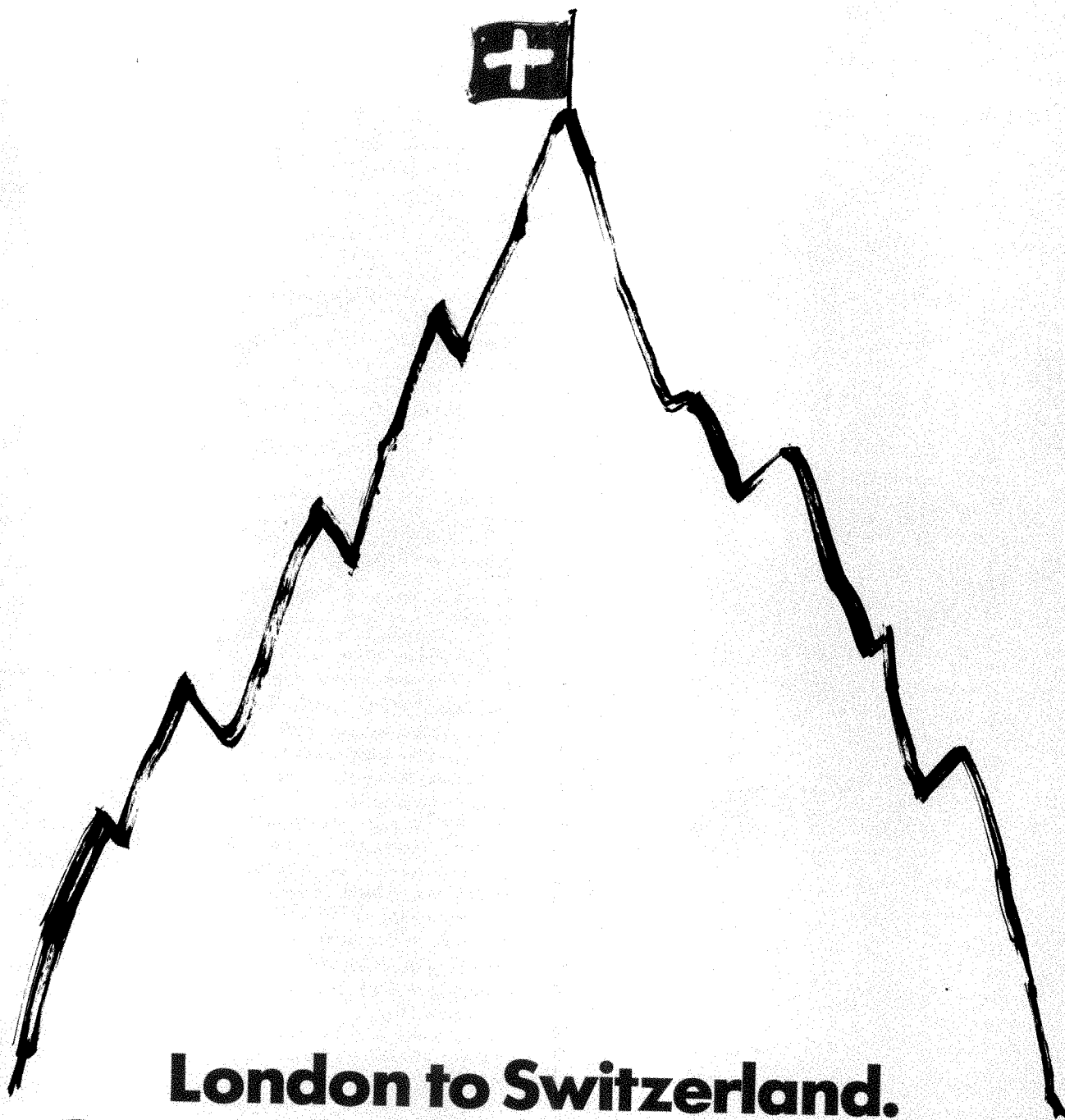
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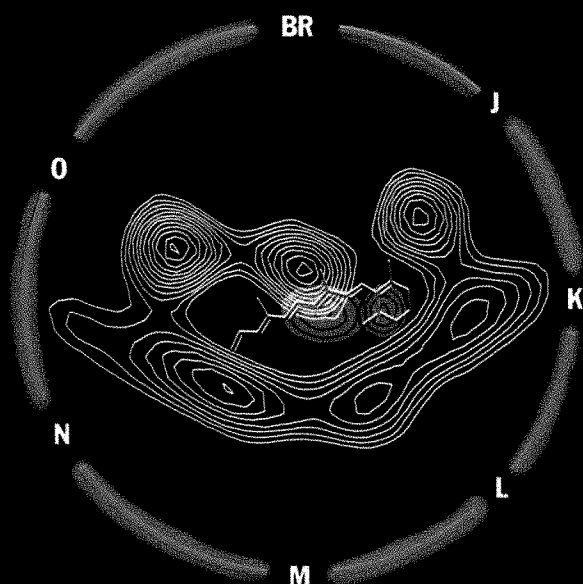
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Picture: Electron density map of Bacteriorhodopsin. Courtesy of  
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## Higher education crisis (cont'd)

Contradictions built into Britain's system of higher education seem to have persuaded the government towards structural change. One ingredient should be freedom — even the freedom for institutions to become extinct.

It is not every day that national higher education systems are augmented by the addition of 25 autonomous institutions, but that is what happened in Britain on 1 April. The provisions in last year's Education Reform Act that replaced the University Grants Committee (UGC) by the untried body called the Universities Funding Council also transferred responsibility for Britain's 25 polytechnics from local education authorities to 25 free-standing boards of governors, whose public support will be channelled through a similar funding council. On the face of things, Britain has for the first time a system of higher education with institutions whose diversity compares with that elsewhere.

This development inevitably marks the beginning of yet another upheaval in British higher education, but one whose consequences cannot be foreseen. But on the face of things, the two sectors of higher education will be differently affected. The universities fear that the new regime will bring even more direct control from the centre: the act gives the government powers to issue general directions to the funding council, which itself has powers to assure itself that the funds which it disburses are spent in ways of which it approves. By contrast, the polytechnics generally welcome the new regime. At the least, they will be rid of the bumbledom of local authority control. Many may even be able to work out for themselves distinctive, even novel, ways of contributing to the higher and professional education of a population increasingly

short of skill. But that is merely how it seems at present. For while the provisions of last year's act are entering into force, the British government seems already to be flirting with even more radical proposals for change.

The self-contradictory decisions of successive governments going back for exactly a quarter of a century (to the then government's enthusiastic endorsement of the Robbins recommendations that the numbers of students in higher education should be increased by a factor of 2.5) have left a hopeless muddle. The decision that the Robbins expansion required the creation of new universities has left Britain with too many universities that are too small justly to claim the title — and which are now being further robbed of academic independence by UGC's otherwise sensible attempts at rationalization.

The decision, just a few years later, that 26 polytechnics (one has since dropped out) should acquire equal status, but be managed differently, may have been most notable as a sign of emerging political animus against the universities, but it created a legacy of division (known as the 'binary system') which even last year's Reform Act left untouched. The same quarter of a century has sanctified the notions that public subventions of universities cover the costs of maintaining a capacity for carrying out research, that eligible students are entitled to maintenance grants (which are nevertheless inadequate for their stated purpose), that tuition fees for home students (now including the EEC) should be way below the economic cost and that academics should be systematically underpaid. Most governments faced with these problems have behaved as if they would prefer to start from somewhere else. The present government, with its reputation for radicalism to preserve, seems now to have woken to the need to cut the knot. After years in which it appeared to be concerned only with the money costs, it now allows that the origins of its difficulties may be structural.

So they are. There is, for example, a glaring contradiction in the university sector between the need that resources should be used effectively and the need that universities should shape the patterns of their own activities. In the first case, UGC prudently, in its last few years, took steps to see that the teaching in fields such as Russian language, agriculture and philosophy should be concentrated at particular universities, chosen (controversially) for their scholarship. A similar reorganization is now under way in the Earth sciences, and there are now pro-

### Computer subscriptions

Even marvellous machines have bugs.

FROM this issue, new arrangements have been made for maintaining and fulfilling subscriptions to *Nature*. Subscriptions in the United States and Canada will be handled by JCI Data Processing Inc. of Delran, New Jersey. For the rest of the world excluding Japan, the maintenance and fulfilment of subscriptions have now been transferred to *Nature's* own computer system equipped with customized VISTA software. While there is every confidence that the end result will be a better service for subscribers, their indulgence for the customary unforeseen problems is earnestly requested. Arrangements for Japan continue unchanged. □



posals that rationalization (as it is called) should be extended to the teaching of physics, chemistry and biology (see *Nature* 338, 363; 30 March 1989). But two foreseen difficulties are now apparent. The cost of reorganization is itself high. And the end-point of the process must be that few British universities will be able to offer a range of studies wide enough to justify their social and academic functions. Is that not a high price to pay for a fudged remedy for mistakes made a quarter of a century ago?

The polytechnics, new-found delight with titular independence notwithstanding, may soon find themselves in a similar case. While their bread-and-butter teaching at the undergraduate level will continue to be supported by their funding council, many of them appear to believe that their future lies in the further development of professional and continuing education, usually of a technical or technological character, for which there is a crying need. More directly than the universities, they will be exploring the now non-existent British market in adult education. The clever among them will no doubt be able to forge profitable links with the network of new technical training councils which the government has separately proposed. But they, too, may then find that the end result is that they have exchanged one kind of thralldom (to local authorities) for another.

The support of research in this changing pattern raises other contradictions. The British research councils make project grants to polytechnics as well as to universities, although the bulk of what they choose to spend in this way ends up in universities. (Part of the explanation is that polytechnic budgets are not reckoned to cover the cost of maintaining research capability, notionally 30 per cent of UGC's recent subventions, which is why they seemed to the Labour government of 1966 to be more "cost-effective".) But since the early 1970s, all the research councils have been spending too great a proportion of their resources on central facilities and too little on research grants, while universities have more recently taken to squirrelling away research money to keep body and soul together. The process has now gone so far that the Science and Engineering Research Council, the largest of the four concerned with science, seems to have put its responsibilities towards individual researchers second after its enthusiasm for directed research (see *Nature* 337, 291; 27 January 1989). So why bother to maintain a capacity for research at universities?

If the British government seriously intends to resolve these issues, it needs some guiding principles. Here is a short list to begin with.

■ **Autonomy.** The British government and its new funding councils must recognize that academic institutions are themselves the most reliable judges of how all kinds of higher education should develop. (Civil servants have a poor track record in this role.) But good judgement requires both the freedom and the size to respond flexibly to changing circumstances. The right to decide what to teach, and how, the cornerstone of academic freedom, is the best guarantor of continuing good sense. Institutions'

dependence on public funds is neither here nor there: the University of California is not noticeably compromised by decisions made by the government of California, which foots the bill. The principle applies as much to the polytechnics as to the universities.

■ **Incentives.** Means must be found of rewarding successful institutions. Much of the present mess in British higher education stems from the way in which institutions have been deprived of a sense of opportunity for close on 20 years. The regulation of capital funds and even of student numbers have been used to constrain the sizes of institutions (keeping too many of them too small). The government should now be prepared to see this relationship turned inside-out. While growth is not everything in academic life, institutions wishing to grow and able to attract the students should be enabled to do so. The other side of that coin is that unsuccessful institutions may shrink, even to the threshold of viability, which may be politically uncomfortable. But how else can a government which believes that market forces discover truth conduct itself?

■ **Research.** Reputation in research is another legitimate goal for academic institutions (and a powerful means by which the quality of its teachers may be generally improved). Research is also, of course, valuable in its own right. But this reward has also, in the past decade, been denied by the scarcity of funds, likely to be further restricted by the research councils' ever-greater fondness for directed research. And in the university sector, there is a prospect that the implicit distribution of research overhead will now be entirely out of pattern with the planned concentration of research. This is why it will seem attractive to the government to arrange for a wholesale transfer of funds of research support in universities from the funding council to the research councils, but that would be a mistake. The more urgent need is to come to a clear decision about the responsibilities of the research councils towards research in both the universities and polytechnics, and then to let institutions of all kinds find their appropriate levels. Less successful institutions may find themselves losing in the competition for survival, but to deny them the opportunity of reversing that trend would be destructive.

■ **Money.** On past form, the British government would probably believe that it had discharged its responsibilities to higher education if it could persuade other people to meet the costs, but the inference is incorrect and the goal absurd. Exhortation will not have British industry or parents supporting higher education the day after tomorrow; even tax incentives on the US pattern would probably have only a small effect (which is no reason for not trying them). But there is a case for changing radically the ways in which funds are at present channelled to institutions. Higher tuition fees (paid for indirectly by central government) are the obvious means by which universities could compete for students and so win back their freedom — even their freedom to go out of business. □

# Cold fusion causes frenzy but lacks confirmation

- Worldwide attempts to reproduce results
- \$5 million may be given for US research

## Washington, Tokyo & London

REMARKABLE reports of "nuclear fusion in a test-tube" achieved by two laboratories in Utah have prompted a frenzy of activity in laboratories around the world as others try to replicate the experiment.

First news of the reported breakthrough in 'cold fusion' came from a press conference on Thursday, 23 March, at the University of Utah in Salt Lake City. Stanley Pons, a professor of chemistry at Utah, and his colleague Martin Fleischmann from the University of Southampton reported that they had achieved fusion by passing a current through a palladium electrode in an electrolyte solution in deuterated water. By the next day, on the basis only of newspaper accounts of the experiment, laboratories around the world were scrambling to copy the experimental design.

At the same time, traffic started flying across computer networks as interested chemists and physicists sought details of the experiment. By the end of last week, preprints of papers prepared for publication by both Pons and a second Utah group, led by Steven E. Jones of Brigham Young University in Provo, were available on electronic bulletin boards.

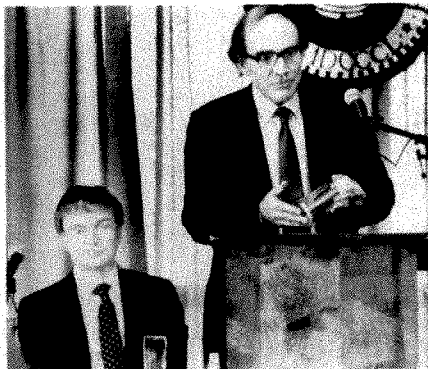
Numerous laboratories in the United States have attempted to replicate the findings. Los Alamos National Laboratory, Lawrence Livermore National Laboratory, IBM in Yorktown Heights, New York, AT&T Bell Laboratories in New Jersey and the University of Illinois at Champaign-Urbana are just some of the institutions that are said to be looking into the Utah claims, although none has reported unequivocal confirmation. The price of palladium has reached new heights on commodity markets.

Professor Noboru Koyama of the Tokyo University of Agriculture and Chemistry reported at a meeting of the Chemical Society of Japan on 1 April that his team had detected very large amounts of heat and gamma rays in an experiment two days earlier when they passed an electric current between palladium and platinum electrodes in an insulated tube filled with heavy water. However, Japanese experts who have seen Koyama's results expressed scepticism about the validity of the claim because no measurements of neutron yield were made. Koyama says he will try to duplicate the experiment with the Japan Atomic Energy Research Institute to determine neutron yield.

In Britain, teams from the University of

Birmingham and the Rutherford Appleton Laboratory and the UK Atomic Energy Authority at Harwell are attempting to confirm the results, and two scientists at the University of Debrecen in Hungary, Gyula Csikai and Tibor Staricskoi, claim to have obtained the same results as Fleischmann and Pons, but no details of their experiment are yet available.

In Italy, the Ettore Majorana Centre for Scientific Culture has announced a



Pons and Fleischmann: handling it carefully.

world conference for next week (12 April) in Erice to discuss the cold-fusion experiments. Europe, the Soviet Union and the United States are about to launch a ten-year, \$1,000-million project to study the viability of fusion by inertial confinement using X-ray lasers, but all may change pending the conclusions of the conference.

Press interest, as well as interest from the scientific community, remains at a fever pitch. At a seminar last week at the Salt Lake City campus, Pons presented additional details of the experiments to a packed lecture hall. He reported that his laboratory had achieved even greater energy efficiency from the experimental apparatus, although he joked that it would be 100 years before the technology could be made commercially available. Pons warned those trying to replicate the experiment to be extremely careful, as his own early attempts had led to heat generation that had run out of control.

The Brigham Young group has been less forthcoming in public about their results. They believe they are seeing a signal indicating that the deuterium atoms are fusing inside the palladium lattice, although they have not seen the same energy output recorded by Pons and his colleagues.

The US Department of Energy (DoE) has been interested in research in cold fusion for some time. According to a DoE

spokesman, the agency has spent some \$9 million in this field, with \$1.9 million going to Jones. A proposal from Pons submitted last fall for DoE support for a project to "further explore nuclear fusion reactions in deuterium-charged palladium" was approved by DoE's Advanced Energy Projects Division, and \$322,000 is expected to be released for the project later this spring.

The state of Utah has shown even greater enthusiasm. The day after the University of Utah press conference, Governor Norm Bangerter announced he would convene a special session of the state legislature for the sole purpose of considering a supplementary budget request of \$5 million for a new institute to pursue research in cold fusion. National Aeronautics and Space Administrator James Fletcher has agreed to sit on an advisory panel for the new institute once it is created. A spokesman for Bangerter says the new money is contingent upon confirmation of the Pons experiment, but it is unclear whether that will come before the legislature holds its session tomorrow (7 April). □

## AUSTRALIA

### Human embryo experiment banned

#### Sydney

THE state government in Victoria has overturned a decision to permit a controversial experiment on human embryos and one member of the committee to regulate *in vitro* fertilization (IVF) has resigned in protest. The experiment, designed to test human embryos for birth defects before implanting them in patients, would have been the first to involve embryos older than 22 hours. Dr John Henley, who resigned, is a theologian and master of one of Melbourne University's colleges. He has been a member of the Standing Review and Advisory Committee on Fertility, chaired by Monash University's Professor Louis Waller, from its inception.

The experiment was to have been carried out by the IVF research group at Monash University, headed by Dr Alan Trounson. It would have involved the biopsy of 11 embryos that had been slow to grow. Trounson says that the experiment was approved unanimously by the committee. "It even passed without comment from Father Harmon (the representative of the Roman Catholic Church), who is not allowed to approve of anything."

According to Trounson, the government's intervention came in response to public pressure, provoked by newspaper reports saying that the experiment would lead to genetic engineering and cloning. He says that "the government's action is an unfortunate politicization of the whole exercise".

Charles Morgan

## Release of sea-floor maps

### Washington

THE US Navy made a U-turn last week and announced that it will permit the release of most of the detailed sea-floor maps of the US Exclusive Economic Zone (EEZ) prepared by the National Oceanic and Atmospheric Administration (NOAA). The announcement follows rumours that the Navy was steaming in the other direction and was about to condemn all high-resolution bathymetric data collected in federal programmes to the same fate as the NOAA data, classified by a White House memo from Vice Admiral John Poindexter in 1985.

The change in policy comes as a welcome relief to oceanographers who were living "under threat that our data might become classified and that we would not be able to freely distribute our results" according to Ken Macdonald of the Marine Science Institute at the University of California at Santa Barbara. The NOAA maps are likely to be an exciting data source, he said, providing detailed information on many scientifically interesting areas off the west coast.

Restrictions on the NOAA maps will not be lifted for all of the 130,000 square kilometres of sea bed they cover. Excluded will be areas, of yet undefined size, around ballistic missile submarine's home ports. The Navy's own massive collection of high-resolution bathymetric data will definitely remain classified. Most of it is believed to be for the deep oceans (the location of surveyed areas is also classi-

fied) and would be of enormous value to oceanographers. But the Navy argues that it needs the data kept secret as it uses it to determine the best sites for sensors to detect enemy submarines and to map the gravity anomalies which affect the flight of ballistic missiles. Occasional glimpses of part of the Navy data have been given on a "need to know" basis, producing, for example, the map of the North Atlantic sea mounts published by David Epp and Christian Smoot (see *Nature* 337, 254; 1989). But even in this case the location of the sea mounts was not given with full accuracy.

The Navy's tough policy on bathymetric data has done little good to US industry. General Instrument Corporation, the major US maker of multibeam sonar, has had to refuse several export orders, while Finnish, German and Norwegian companies have sailed away with business. The damage to US industry, plus the knowledge that it was becoming possible to buy foreign sonars that would map the sea floor as accurately and faster than NOAA's equipment, may have helped to prod the Navy into a more open attitude.

Most oceanographers attribute the change in policy to last year's appointment of Rear Admiral Richard Pittenger as Oceanographer of the Navy. Despite his background in anti-submarine warfare, he is believed to value academic research. But the Navy keeps quiet about its policy decisions and will not give reasons for its change in course.

**Alun Anderson**

### RADIOTELESCOPE

## Green Bank failure identified

### Washington

THE cause of the sudden collapse of the 300-foot radiotelescope at Green Bank, West Virginia, last November (see *Nature* 296, 336; 1988), on a night without wind, rain or snow, has been traced to the fracture of a single, highly stressed steel connection plate, according to an independent inquiry which submitted its findings to the National Science Foundation last week.

That conclusion leaves the National Radio Astronomy Observatory free to clear away the enormous tangle of steel that had been left undisturbed while the inquiry was in progress. But it makes it no easier to sort out the scientific and political issues surrounding the question of whether the telescope should be rebuilt.

The three-man inquiry panel found no evidence of inadequate maintenance of the connection plate, or of improper operation of the telescope. But modern computer structural stress analyses, show that stresses in many parts of the

telescope "were substantially higher than is permissible today".

The telescope's design problems can be overcome using modern techniques, says the report, and provide no technical obstacles to its replacement. West Virginia's two Democrat Senators, Robert C. Byrd and John D. Rockefeller, are already demanding that a similar, state-of-the-art telescope be built as the "best promise for jobs, education, tourism and scientific prestige" in a state with little else to offer in the way of large, basic research facilities. Byrd, who holds a powerful position in the Senate Appropriations Committee, says he "will aggressively pursue funding" for the telescope. Plans for a supplemental appropriation to the NSF budget for a new telescope are already under way.

But astronomers are not necessarily sure that replacing Green Bank would be the best use of the \$75 million required. The large telescope was valuable for survey and mapping and was able to detect

## Protest as Pasteur speaks English

### Paris

A DECISION to give the Pasteur Institute's journal an English title has caused confusion and protest in the French press, but seems to have left the scientific community unscathed. Under the banner "Goodbye Pasteur!", the French national newspaper, *Le Monde*, broke the news that, in future, the *Annales de l'Institut Pasteur* will be published only in English, for the first time since it was created in 1887. This, ran the article, sounds the death-knell for French-language science.

Two changes have been made to the journal. The title will now be *Research in ...* (immunology, virology or microbiology, according to the discipline covered). Second, the review panel has been widened to include non-Pastorians and foreign scientists. But, says the Pasteur Institute, articles submitted in French and accepted for publication will be printed in French, while articles in English will continue to have a French abstract. For the press, the changes signal the disappearance of a flagship of French science under the 'tyranny' of the English language. But the Pasteur Institute sees things differently. "In 1973 only 10 to 15 per cent of the articles we received were in English, but in 1987 the proportion was almost 100 per cent", says a spokeswoman. "But of the articles we published, 58 per cent were from French-speaking authors and over 80 per cent of these were originally written in English. The title of the review, however, suggested that it was not open to the scientific community as a whole and so researchers started to send their papers elsewhere. The *Annales de l'Institut Pasteur* has to survive."

**Peter Coles**

faint sources, including a pulsar in the Crab nebula. But according to Kurt Riegel of NSF's Division of Astronomy, a decision to rebuild the telescope would face competition from projects to upgrade the radiotelescope at Arecibo, to build large optical telescopes and to enhance the image-processing capabilities of the National Radio Astronomy Observatory's very large arrays.

If a new radiotelescope is not built, "it does not bode well for the site at Green Bank", according to the facility's director, Paul A. Vanden Bout. A 140-foot radiotelescope remains at Green Bank but it is 26 years old. A possible solution, already suggested by NSF officials, is to build the eastern end of the proposed Laser Interferometer Gravitational Wave Observatory (LIGO) at Green Bank. The project requires a facility in California and in the east to be run in coincidence. But Byrd and Rockefeller say that while they welcome LIGO, they want the radiotelescope replaced too.

**Alun Anderson**



## CANCER THERAPY

## Cancer data are puzzling

### Washington

THE US General Accounting Office (GAO) has uncovered a conundrum in breast-cancer therapy. Although there is good evidence from randomized clinical trials that chemotherapy following surgery is beneficial for premenopausal women with breast cancer that has spread to the lymph nodes, increased use of chemotherapy has not been followed by decreased mortality statistics. In 1987, Congress asked GAO — its investigative agency — to determine whether cancer patients were actually receiving the latest therapies being developed with federal money by the National Cancer Institute. In its latest report\*, GAO used data from the Surveillance, Epidemiology and End Results (SEER) programme to track mortality from breast cancer from 1975, when chemotherapy was first shown to be an effective treatment, until 1983, the latest year for which complete data are available.

Although there is still some debate over the value of chemotherapy for breast-cancer patients whose cancer has not spread to the lymph nodes, clinical trials have shown that for node-positive premenopausal women, chemotherapy is beneficial. Nevertheless, despite an increase in the number of women receiving chemotherapy between 1975 and 1983, there is no detectable change in survival figures. The analysis confirmed that age and race are significantly related to the probability of dying from breast cancer.

GAO proposes three explanations for its results. The first, and least likely, is that chemotherapy is not so beneficial despite studies to the contrary. A second possibility is that doctors are not giving appropriate or sufficient drugs to their patients, and a third possibility is that the improvement that can be expected from chemotherapy is not great enough to be detected by GAO's statistical techniques.

But Vincent DeVita, Physician-in-Chief at Memorial Sloan-Kettering Cancer Center and former director of the National Cancer Institute, says GAO is premature in raising the long-term survival issue. He argues that only a small fraction of the appropriate patient population was receiving chemotherapy in 1975, and newer therapies will yield a more encouraging picture in a few years. DeVita accuses GAO of designing its study to validate preconceived ideas.

The GAO report calls on the Department of Health and Human Services to conduct a larger study to explain the curious findings.

**Joseph Palca**

\* Breast cancer: patients' survival. United States General Accounting Office, GAO/PEMD-89-9, Washington, DC, 1989.

## RESEARCH ANIMALS

## Activists infiltrate Stanford

### Berkeley

THE Stanford University biology faculty has become involved in a new controversy over the use of animals in research by criticizing an undergraduate course taught by two animal rights activists. A letter from the biology faculty objecting to the course was obtained by the two instructors, who are now demanding an apology.

Stanford has had difficult relations with animal rights activists for the past several years. Protesters have caused more than a year of costly delays for Stanford in building both a new animal facility and a new biology research building (see *Nature* 329, 477; 1987). So the biology faculty was dismayed to learn last autumn of a Stanford course called "The Case for Animal Rights", being taught by Kimberly Sturla



and Lise Giraud of the Peninsula Humane Society, both of whom have been involved in the building protests.

The course was part of a popular programme called Stanford Workshops on Political and Social Issues (SWOPSI), intended to expose students to controversial subjects. Biology chairman Philip Hanawalt says department members did not object to a course on animal rights, but were concerned because one of them who had attended the class reported that the instructors showed ignorance of the scientific arguments for using animals, and created a hostile environment for speakers invited to present that view. The faculty voted unanimously that a letter of objection should be sent to the dean of undergraduate studies.

"We decided that it is the prerogative and duty of the academic faculty to maintain quality control in what is taught to Stanford students", said Hanawalt, "and therefore it was entirely appropriate for us to make comments on this." Those who observed the class and wrote the letter have requested anonymity out of fear of reprisals by the activists.

The letter, which was addressed to

Thomas Wasow, dean of undergraduate studies, with a copy to Margo Horn, director of SWOPSI, questioned the qualifications and scientific knowledge of the instructors, and accused them of requiring ideological compliance of the students and of failing to present both sides of the issue. When the instructors heard of the letter and asked for a copy, Horn consulted Wasow, who agreed that she could send them one. Wasow says that at the time he did not consider the letter, which bore the names of the entire biology faculty, to be confidential. Hanawalt says such internal memoranda should be considered confidential. He compares its release to the instructors to the release to a faculty member denied tenure the letters upon which that decision was based.

Through their attorney, Sturla and Giraud have publicized the letter, called it "defamatory" and demanded an apology. Sturla claims she is "very qualified", to teach the course, and points out that the course dealt with issues other than research, such as the use of animals for food and in rodeos. She adds that in each case, experts were brought in to present each side of the argument.

David Maurice, a professor of ophthalmology who spoke in favour of animal use in research, says he was given a polite hearing, but felt that his words fell on deaf ears, as the students had already been strongly biased against animal use. Maurice, who himself co-taught a seminar on alternatives to animal use several years ago, questions whether activists like Sturla and Giraud are the right people to teach such a class.

**Marcia Barinaga**

## SCIENCE POLICY

## Brazilian appointment

### São Paulo

CONTINUING its quick-change approach to science and technology policy, the Brazilian government last week selected a secretary for its new Special Secretariat for Science and Technology, a cabinet-level body created just two months after the sudden extinction of the Ministry for Science and Technology. The appointment of Décio Leal de Zagottis, director of the influential Polytechnic School of the University of São Paulo, may signal a change in computer-industry policy. In the two months following the death of the old ministry, science policy and the management of several government institutes went to the Industry and Trade Ministry, whose minister, Roberto Cardoso Alves, opposes Brazil's protectionist stance towards the computer industry. The new secretary says he will oppose change.

Ricardo Bonalume Neto

## Proposal divides biologists

### London

BRITAIN'S biology community is divided on the merits of the new plan to restructure biology teaching and research in universities (see *Nature* 338,363; 30 March 1989). Some are resigned to the plan, others will strive to persuade the Universities Funding Council (UFC) to modify some of the proposals.

The report, distributed to universities

last week, was carried out for the University Grants Committee (which was dissolved last week) by a group led by Professor Sir Richard Southwood of the University of Oxford. It was the subject of heated discussion at the first meeting on 31 March of a new group comprising more than 50 heads of biology departments.

The most controversial aspect of the plan is the proposal to group existing

departments into two kinds of much larger departments, one focusing on molecular biology and the other on more traditional areas such as ecology and evolutionary studies. This was criticized as "disastrous", "illogical" and "an odd reversal of the current trend towards integrative biology". One speaker said it would divorce the techniques of molecular biology from the subject areas in which they were being applied. The department heads agreed that a rigid division between the two types of department would be detrimental.

On the other hand, they disagreed over interpretation of the report, some saying it was too dogmatic and others insisting that there would be flexibility.

The proposal to group all biology departments in a university into a single school of biological sciences was also welcomed. Most agreed that fragmentation is undesirable, but the report was criticized for putting forward only one model for integration. The group agreed that for different universities, different strategies would be necessary.

The group also agreed with the recommendation that pre-clinical teaching of biology in schools of medicine, dentistry and veterinary science should be the responsibility of the new schools of biological sciences. Professor James Callow, of the University of Birmingham, described this as the "most radical proposal" in the plan, but the review committee was criticized for not studying this problem in more detail. This proposal is likely to meet fierce opposition in medical schools.

The biology heads were particularly concerned about the effects on biology of the planned restructuring of other sciences. Although the biology plan recommends no closures, the expected closure of more than ten university chemistry departments is expected to have a knock-on effect on biology.

There was also concern about the future quality of entrants into university biology courses. Changes in the content of school examinations are likely to mean that students have less factual knowledge in the sciences. The biology heads also fear that the courses provided at British universities may compare badly with more comprehensive and longer courses at other European universities. One proposed solution to both these problems was that England should follow Scotland in introducing 4-year degree courses, instead of the present 3-year courses.

The committee elected by the new group of heads will put a response to the biology report formally to the new Universities Funding Council later this month. This report was intended to be only the precursor to a more wide-ranging review, but was carried out quickly so that restructuring of biology would not lag too far behind restructuring in the other sciences.

**Christine McGourty**

### SOVIET SPACE

## Phobos failure raises doubts

### London

THE failure last week of Phobos-2, the second of the two Soviet Mars probes launched last July, could hardly have been worse timed for Soviet space planners. During the past few months, there has been growing public criticism in the Soviet Union of the cost of space, particularly of deep-space missions with no obvious economic spin-off. One of the chief advocates of swingeing cuts in the space budget is Mr Boris Yeltsin, who on 26 March won a resounding election victory over the 'official' candidate. The following day, contact with Phobos-2 was lost.

The two-craft Phobos mission included a wide range of investigations of Mars and its satellite Phobos, including the firing of a laser at Phobos and the landing of a 'hopping' probe to investigate the satellite's surface. It carried experiments from 13 countries and multinational agencies and had been presented as a first step to an international manned mission to Mars early in the next century. But Soviet media coverage of the mission has from the beginning been tentative.

The original mission consisted of two spacecraft, carrying different experiments. Early in September, Phobos-1 came to grief when a ground-based computer error led to its being sent an incorrect command. In December, the main transmitter on Phobos-2 failed, but the craft arrived on schedule and on 23 January was inserted into an orbit around Mars. It functioned as planned for the next nine weeks, returning several photographs of the surface of Mars and Phobos.

On 27 March, the command to rotate the probe to prepare for the close (50-m) transit of Phobos was apparently followed correctly — but mission control found it impossible to re-establish contact. Two days later, the head of the Glavkosmos space agency said his experts were working round the clock to try to restore the links. But these efforts proved fruitless.

Although Soviet newspapers have made the best of the photographs and data already received from Phobos-2, these are unlikely to restore public confidence. But

as *Pravda* revealed last week, there is equal resentment that it will be a Japanese journalist (paying his fare in hard currency) and not a Soviet citizen who will be the first media representative to visit the Mir station (see this page).

**Vera Rich**

### JAPAN IN SPACE

## Journalist plans scoop

### Tokyo

THANKS to the Soviet Union, the first Japanese citizen to journey into space may be a journalist from a private television network rather than one of the astronauts training for a ride on the US space shuttle.

Last week, the Tokyo Broadcasting System (TBS) network signed an agreement with the Soviet Commission for Space Exploration (Glavkosmos) that will allow a TBS reporter to spend six days aboard the Mir space station in 1991. The reporter will make live broadcasts to Japan every day throughout his stay in the station.

The agreement comes as a shock to Japan's National Space Development Agency (NASDA), which has been planning for years to put the first Japanese astronaut in space aboard the US space shuttle. Although a final date for the reporter's space voyage has yet to be set, TBS has arranged the flight to commemorate the network's 40th anniversary on 10 May 1991. There is thus a strong possibility that TBS will 'scoop' NASDA's plans to put an astronaut on the space shuttle in July of the same year.

Soviet journalists are also upset about the agreement. *Pravda*, the Communist party daily newspaper, welcomed the Soviet authorities' decision to offer a journalist a space flight but complained that only foreigners could afford to pay the fare. The Communist Party youth newspaper *Komsomolskaya Pravda* carried, under the headline "Prestige trampled underfoot", pictures of Yuri Gagarin, the first man in space, Valentina Tereshkova, the first woman in space, and a Japanese 10,000-yen banknote with the caption "The first journalist in space?".

**David Swinbanks**

## OIL SPILL

# Shipwreck fouls the water

✓ **Berkeley**

THE largest oil-tanker spill in US history has turned Alaska's Prince William Sound from a pristine environment into a disaster area. Biologists are not yet able to assess the full extent of the damage to the area's wildlife, but the political damage to those pressing for further oil development in Alaska is already plain.

On 24 March, the fully-loaded oil tanker *Exxon Valdez* struck a reef shortly after leaving the Alaskan pipeline terminal in Valdez, southern Alaska, spilling 10 million gallons of crude oil. The tanker's captain, who had been drinking before the accident, had left the ship in the hands of a crewman not certified to pilot the ship within the sound.

Little oil from the spill was recovered, and angry fishermen and environmentalists blame Exxon and the Alyeska Pipeline Service Company, which manages the pipeline, for a disorganized clean-up effort that squandered precious time. Floating booms intended to be deployed quickly to contain a spill did not reach the site until more than 10 hours after the wreck, said Jon Lyman, of the Alaska Department of Fish and Game. Alyeska officials blamed the delay on the fact that the barge intended to carry the booms was unloaded for repairs.

Alyeska spokeswoman Beverly Michaels said that skimmers and booms are not practical for such a large spill, and Alyeska was prepared on the day after the spill to apply chemicals that disperse the oil into the water. But fishermen, concerned about the toxic effects of the dispersants and dissolved oil, persuaded the Coast Guard to delay permission to use them. By the time permission was received the following day, said Michaels, high winds made it impossible to apply the dispersant from aircraft. Debate still continues over whether dispersants or containment and collection would have been best, but after two days of heavy winds the oil was spread over 550 square miles, ruling out either method. Fishermen and state officials joined Exxon and Alyeska in using barriers to protect the most environmentally sensitive areas.

Because the Valdez spill is in a relatively enclosed area, it formed a continuous blanket over the water, potentially trapping aromatic hydrocarbons, such as benzene or toluene, in the water beneath the oil. Jacqueline Michel, an environmental chemist for the National Oceanic and Atmospheric Administration (NOAA), said laboratory studies have shown such chemicals to be highly toxic to planktonic fauna.

The herring-roe fishery will be the first influenced by the spill. Within the next few weeks, the fishery would normally

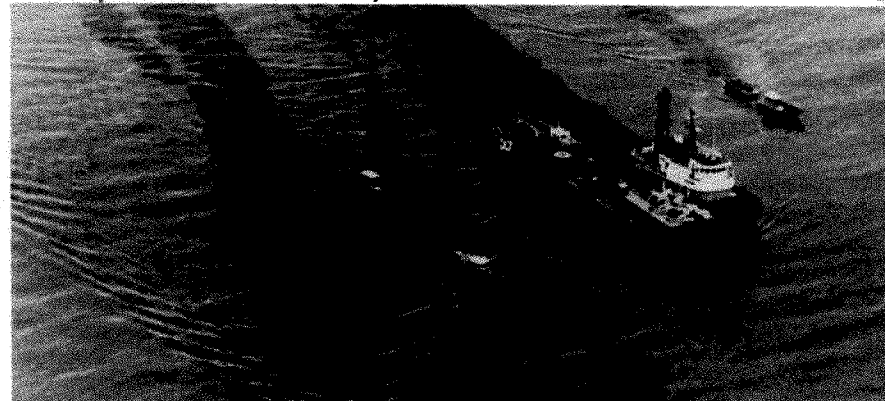
begin to capture herring that have entered the sound to spawn, and allow them to deposit their eggs on kelp, which is harvested after the fish are released. Sale of this year's harvest may be banned if hydrocarbon contamination is detected in the spawning areas or in the roe that is collected.

High priority was given to protecting the economically important hatcheries where young salmon are raised and returned to the sea in May. Some are tagged each year before release, and survival data are collected when the adults are caught by fishermen several years later. Tags from those released this year will provide information on the impact of the spill on the salmon population.

Lyman said that birds and sea otters are particularly vulnerable to oil spills, because the oil destroys the insulating qualities of their fur and feathers. Exxon brought in an animal rescue team from California, but rescue attempts were hindered by the inaccessibility of the rocky beaches in the area. Tens of thousands of birds and otters live in the sound, and many of those are likely to have been harmed or killed by the oil, but a week after the spill only a handful had been rescued and cleaned. The cleaned animals will be tagged before their release, to aid biologists in determining the fate of animals following the spill.

Migratory puffins, terns, cormorants and other birds are just beginning to enter the area. Their nesting grounds, as well as the areas where sea lions will give birth in May, were included among areas to be protected with booms from the progressing oil slick.

Exxon will hire local residents to clean oil from hundreds of miles of shoreline. Exxon spokesman Brian Dunphy said absorbent pads will be used to soak up the oil, and high-pressure hoses will blast it off rocks. Michel, of NOAA, said wave action and biological activity will purge the oil from exposed sites, but sheltered areas may remain contaminated for years.



Barges and tugs head to clean up the worst of the oil spill in Alaska's Prince William sound, where the *Exxon Valdez* ran aground spilling 270,000 barrels of crude oil.

## NUCLEAR SAFETY

## Cuba signs agreement with Soviet Union

London

CUBA has signed a nuclear safety agreement with the Soviet Union, regulating Soviet assistance at the Cienfuegos nuclear plant and "other nuclear projects" under construction. The Soviet side is to equip Cuban nuclear safety laboratories with services, hardware and documentation, and will also arrange consultancy services on how to organize radiation monitoring. Cubans will be trained in the Soviet Union as operators of nuclear power stations.

Vera Rich

The spill is likely to cause political fallout, as the battle continues in Congress over oil development in the Arctic National Wildlife Refuge, adjacent to the Prudhoe Bay area which now supplies oil to the Alaskan pipeline. Environmental groups have opposed the development (see *Nature* 327, 9; 1987), while oil companies and the Bush administration have argued that development is essential, and can be conducted in an "environmentally sensitive" manner. The Valdez spill did not change administration policy, but environmental groups expect its effects on public opinion to be felt in Congress, where competing bills are being considered that would either open the area for development or declare it a wilderness area, thus preventing oil drilling. Opponents of oil development off the California coast expect the spill to aid their cause as well.

Although an oil spill at the pipeline terminal is not the same as the general environmental disruption feared from construction of oil exploration sites in the Alaskan wildlife refuge, Don Hellmann, of the Wilderness Society, said it raises the question of whether assurances from oil companies can be trusted. "The oil companies have been saying all along that they can drill without harming the environment, and if there is a problem such as a spill, they can take care of it right away. Now we've seen that they're wrong on both counts."

**Marcia Barinaga**

Associated Press



## Operators to cooperate

### Boston

NEXT month in Moscow, the World Association of Nuclear Operators (WANO) will begin formal operations, ushering in what supporters hope will be an era of increased international cooperation among the world's nuclear power industries.

WANO was conceived initially in response to the accident at the Chernobyl reactor in 1987. Participating power plants will share information about the safe operation of their plants and the details of any "off-normal" events through a worldwide computer network. The association has already opened regional centres in Paris, Tokyo and Atlanta (Georgia), and a coordinating office in London. A fourth is due to open in Moscow later this month. In a remarkable show of solidarity, the organization will include the participation of every nuclear utility in all of the 31 countries that use the technology to generate electricity.

Through its regional centres, the group will collect data about all mishaps and untoward events at nuclear power plants, and will organize workshops and technical exchange visits between nuclear operators from different nations.

While other international organizations exist to promote the safe use of atomic energy, WANO is unique in its exclusive orientation to industry, and its goal of uniting nuclear-plant operators worldwide. WANO is modelled on an existing organization in the United States called the Institute of Nuclear Power Operations (INPO) which was also formed in response to a nuclear mishap—the partial meltdown at Three Mile Island in 1979 (see *Nature* 338,190;1989).

Greater information exchange among nuclear operators might have prevented the operator error that led to the accident at Three Mile Island, according to many INPO staff. Addressing participants at an INPO conference in Atlanta last fall, Lord Marshall, chairman of the WANO Steering Committee, stated that the accident at the Chernobyl reactor "never would have happened" if WANO had been in place and "working effectively". "Peer group pressure" would have forced changes in the "unacceptable" degree of reliance on the operator in the safety design of the Chernobyl plant, said Thomas Eckered, acting director of the London WANO coordinating centre.

Marshall stressed the importance of the choice of Moscow as the location of the inaugural meeting of the group. That, he said, shows the acceptance by the world nuclear industry of Soviet commitment "to *glasnost* and *perestroika* in their nuclear business."

**Seth Shulman**

## Proliferation of nuclear subs

### Boston & São Paulo

FEARS that the acquisition of nuclear-powered submarines by Brazil, India and Canada will blur the line laid out in the Nuclear Non-Proliferation Treaty and foster the spread of nuclear weapons to developing nations are largely unfounded, according to the majority view at a meeting held last week at the Massachusetts Institute of Technology (MIT). But military and technical experts from several nations, including those planning to acquire nuclear-powered submarines, agreed that there are real fears that deployment of nuclear submarines could trigger regional arms races in South Asia and South America.

Brazil, India and Canada are all planning to build or buy nuclear-powered submarines. India last year leased a nuclear submarine from the Soviet Union and is rumoured to be negotiating for three more, even as it continues work on its own development programme. K.K. Nayyar, retired vice-admiral of the Indian Navy, defended his nation's nuclear-submarine programme not only by emphasizing its deterrent capability but also by acknowledging India's desire for a stepped-up military presence in the Indian Ocean. "India represents 17 per cent of the population of the planet", Nayyar stated, "we want to be there to be reckoned with".

Brazil too has regional power plans. A statement from Mario Cesar Flores, an admiral in the Brazilian Navy, said that

Brazil's ambition to have a "strong presence" in the South Atlantic leads it "in a firm, prudent and deliberate fashion, to the nuclear-powered submarine". Brazil was apparently impressed by the performance of British nuclear submarines during the Falklands war.

Three conventional submarines are now being built in Brazil with West German help while indigenous efforts are made to develop small reactors and enrich nuclear fuel at the Centro Experimental Aramar at Iperó near São Paulo. According to Adrian English, a US expert on Latin America's military balance, as soon as Brazil deploys a nuclear-powered submarine, "two to four nations in the region will be sure to follow suit", including Argentina, Chile and Cuba.

The role of nuclear-powered submarines in regional conflicts seems a bigger problem than their impact on nuclear-arms programmes. Several participants at the meeting pointed out that the countries currently investigating the development of nuclear submarines already have nuclear-power programmes and "plenty" of plutonium for weapons at their disposal. Brazil's Admiral Othon Pihheiro, known locally as the "Brazilian Rickover", claimed success for the nuclear enrichment programme last week, saying that the first batch of enriched uranium reactor fuel will be delivered at the end of the year.

**Seth Shulman & Ricardo Bonalume Neto**

### ENVIRONMENTAL PROTECTION

## Trouble at the mill in Australia

### Sydney

PLANS for a \$1,000-million chemical pulp mill in Tasmania have been scrapped after the Australian government decided to impose strict environmental controls as a condition of approval for foreign investment in the project. The mill, at Wesley Vale, was to have been a joint venture between the Australian company North Broken Hill Peko and its Canadian partner Noranda.

Such environmental guidelines are usually set by the state government. It is believed that the Tasmanian government agreed to loosen its guidelines because of the employment opportunities offered by the project. But in this case, because of the level of foreign investment, the project had to be approved by the federal government's foreign review board.

At issue is the production of dioxins, which occurs when mills bleach paper with chlorine. In humans, dioxin exposure causes the skin disorder chloracne and has been linked to other illnesses. According to government sources, the companies involved in Wesley Vale were not prepared

to spend enough money to replace dioxins in the bleaching process, or to change the mill's effluent pipeline into Bass Strait.

The Minister for the Environment, Senator Graham Richardson, has come under strong pressure from the government to develop clear-cut environmental guidelines. While agreeing to do so, Richardson has stated that differing circumstances in individual cases may make them difficult to formulate.

Companies at present planning three other pulp mills in Victoria, New South Wales and West Australia, all similar in scale to Wesley Vale, are unlikely to proceed without comprehensive guidelines.

Prime Minister Bob Hawke told the Australian Broadcasting Corporation radio programme "PM" that he recognized that the loss of the mill would damage both investment in Australia and export earnings. "As long as I'm Prime Minister I won't have development at any price. Each day they would be dumping 13 tonnes of organochlorides into the ocean and I will not allow it."

**Tania Ewing**

# Coming of age in anthropology?

Adam Kuper

With the screening in several countries of a film on the subject, the controversy over Margaret Mead's work in Samoa rumbles on. Underneath the thunder and lightning, considerable anthropological issues are at stake.

*MARGARET Mead and Samoa*, a new Australian film made for television, is the latest engagement in a controversy which began in 1983 with the publication of Derek Freeman's book, *Margaret Mead and Samoa: The Making and Unmaking of an Anthropological Myth* (Harvard University Press). The film has been shown in Australia, the United States, Denmark and Sweden; it is presented not just as a journalistic review, but as a contribution to the debate in its own right, a claim which Derek Freeman endorses in person. Indeed the promise is that matters will be settled once and for all — the film opens with the dramatic announcement that the issues in contention "will be resolved by startling new evidence presented in this programme".

But what is all the fuss about? Why did a critical book on Samoan ethnography stir up such excitement? The public has been led to believe that if Mead is shown to be wrong about adolescent girls in Samoa, this will have decisive implications for debates about how far our capacities and characters are shaped by experience, especially childhood experience, and how far they are fixed by heredity. These are some of the greatest issues which have exercised social scientists in the twentieth century. In the manner of the cinematic genre, however, let us backtrack and recall the story so far...

Mead's study of Samoan adolescent girls was carried out in 1925, when she was 23 years old. It was reported over 60 years ago in *Coming of Age in Samoa* (William Morrow, 1928), a short book written in popular style. At her publisher's suggestion, Mead added a final chapter which drew a moral from Samoa for educationists in the United States, and her book became a hugely and enduringly successful best-seller. She also wrote a sober ethnography of Samoa, *Social Organization of Manu'a*, which was published by the Bishop Museum in Hawaii, and together with various collaborators she later carried out more sophisticated studies of other Pacific communities.

Nevertheless it is this famous apprentice book which Freeman chose to try and refute more than half a century later, identifying it as the cornerstone of relativist, anti-biological social science.

Mead spent nine months in Samoa, but her main study of 50 girls in one village was carried out in a period of four months,

whether adolescence was necessarily a period of stress and discord, caused by the ineluctable biological processes of puberty, or whether the problems that American adults experienced with teenagers could be put down to specific cultural factors, and so, perhaps, ameliorated or even eliminated by making changes in schools or in family life. According to Mead, adolescent girls in Samoa had no problems of sexual adjustment, enjoying a fulfilling and undemanding love life. They seemed to have an untroubled passage to maturity. There were few sulky, angry adolescent rebels. Adult Samoans were also relaxed, tolerant and genial. Emotions seldom ran high and there were no intense attachments, even within the immediate family. The society was remarkably free of violence and aggression. The conclusion was that the storm and stress of American adolescence was a culturally specific phenomenon, and had to do with American attitudes to sex and to competition, and to the intense pressures of family life.

Freeman disagrees with most of Mead's ethnographic generalizations. He says, for example, that there is strong mother-child bonding in Samoa, that parental discipline is severe, and that there is a high level of general aggression and violence. Adolescent girls do not enjoy sexual freedom; the Samoans put a premium on virginity, but rape is common, and because of the emphasis on chastity a girl may actually be forced to marry her assailant.

The research for Freeman's critique of *Coming of Age in Samoa* was begun in the 1940s, and his own book was substantially completed by the 1960s. Unfortunately it appeared only some two decades later, several years after Mead's death. But although we are denied Mead's response, a number of regional specialists have assessed Freeman's book in some detail.

Did he successfully refute Mead's conclusions? The consensus among anthropologists is that both Mead and Freeman over-generalized in characterizing the 'ethos' of Samoan culture; in any case,



Duped? Margaret Mead in 1958.

a very short time by modern fieldwork standards (and her work was badly disrupted by a hurricane). Yet her research was not without merit, despite some obvious shortcomings. She became a confidante of the village girls, and carefully recorded their activities and conversations; she applied various psychological tests; and she systematically collected biographical information.

The main lines of Mead's Samoan study are familiar to generations of readers, including millions of college students in the United States. The central issue was

Cornell Capa

defining national character is a hopelessly subjective and impressionistic project, which most contemporary anthropologists have abandoned. The experience of adolescence in Samoa is a different matter, however, and it should be possible to collect accurate information on such questions as the punishment of children, sexual crime, courtship procedures and other matters on which there has been fundamental disagreement. Yet on issues of this kind, Freeman's own data are by no means uncontroversial. For example, he and his wife carried out a 'virgin census', which established the sexual purity of Samoan adolescents. He does not explain how this was done in a community famous, on his own testimony, for its teasing of investigators.

Freeman is also often uncritical in his use of sources. His statistics on criminal violence in Samoa, for instance, are derived from a mixture of reports from different periods and of diverse provenance. He uses them to construct 'rates' and then makes unsystematic comparisons with juvenile delinquency in American and British cities. (Oddly enough, his main comparative source on youth delinquency is an old study by the notorious fraud Cyril Burt.)

What, then, does the film add to the controversy? The central figure is Freeman himself. He is shown in his garden in Canberra, padding through the woods in gloves, driving what seems to be an armoured personnel carrier through quiet suburban streets, wearing funny woollen hats, dressed as a Samoan and as a naval officer — all the while pressing his charges against Mead and her defenders.

### Leg-pulling

The main thrust of the film is an endorsement of Freeman's account of how Mead went wrong. Freeman has always claimed that Mead was systematically misled by her Samoan informants. In the film a young American anthropologist testifies that Samoans do go in a great deal for leg-pulling. An elderly Samoan chief then reiterates the point that Samoan girls are notorious teasers. Finally, an 80-year-old Samoan lady appears on the screen. She is identified as one of Margaret Mead's informants from 1925, and a voice-over tells us that "her account will settle once and for all the controversy. . ." — yes, she says, yes we lied to her, "we just lied and lied".

Epimenides the Cretan who said that all Cretans are liars posed a famous problem for logicians. Samoans who say that all Samoans are liars discredit those who believe anything they may say. Surely Professor Freeman cannot believe that the testimony of a confessed liar will finally persuade his colleagues that Mead's account of Samoa was wrong?

What of the wider issues? Probably, the

general view among anthropologists is that we are very plastic creatures, and that there is considerable culturally induced variation in personality, values and life strategies. But this 'relativist' orthodoxy has always had to contend with a countervailing search for a bed-rock of 'human nature', even of 'nature', that is shared by all human beings, perhaps all primates. Freeman hopes to establish general propositions about human nature; but he is cagey about whether he expects these to be of the sort proposed by Konrad Lorenz, by E.O. Wilson or even by Sigmund Freud.

Yet, although the battle-lines are clearly drawn, certainly by Freeman and by the media, the debate seems in a curious way to pass them by. Even if Freeman's observations are accurate, and Mead's are mistaken, this would not greatly affect our contemporary view of the adolescent experience. Freeman has not tried to show that ethology or social biology offer a more powerful theory of adolescence than any other on offer. He himself quotes the conclusion of H. Katchadourian (*The Biology of Adolescence*; W.H. Freeman, 1977) that "research on ordinary adolescents has generally failed to substantiate claims of the inevitability and universality of adolescent stress", a judgement which would have discomfited neither Mead nor her supervisor, Franz Boas, half a century ago.

Freeman's alternative claim — which he makes in the name of Karl Popper — is that he has refuted Mead. By disconfirming her Samoan findings, he has kicked away the essential crutch of her cultural relativism. This is to assume that Mead's study of Samoan adolescents, carried out 60 or so years ago, was and remains the crucial experiment for the cultural relativists. The claim has only to be formulated in this straightforward way for its flaws to be apparent.

There is therefore little in the view (fostered by Freeman, and taken for granted in this film) that the Freeman-Mead debate is a crucial round in the battle between cultural relativism and a biologically grounded, scientific, universal view of man. Nor are there serious grounds for the claim, supported by some conservatives, that Freeman has undermined the basis for liberal educational philosophies. Nevertheless, the debate *has* had a special resonance within anthropology, for it has fed the contemporary unease about ethnographic fieldwork and writing.

Doubts on this score trouble anthropologists more than any substantive questions today, for they have invested a great deal in the credibility of their characteristic research methods. It is often said that ethnographic fieldwork is to anthropology what the blood of the martyrs is to

the Church. Many leading anthropologists prefer to call themselves ethnographers, and practically every move an anthropologist makes gains in respectability if it is called 'ethnography'. One goes into the field 'to do ethnography' and comes home 'to write an ethnography', and most professionals firmly believe that the better ethnographies will always be of more enduring value than even the most ingenious anthropological theorizing.

### Participant observation

A revolutionary new way in which to do ethnography, participant observation, was introduced shortly before Margaret Mead sailed to Samoa and quickly became the central procedure of modern anthropology. Earlier methods of data collection had been drawn from other sciences. For example, the first professional — scientific British ethnographic expedition, the Cambridge University Expedition to the Torres Strait, in 1898, was conducted by natural historians in the manner of a zoological field study. The natives were observed and their responses tested; and although they were certainly allowed to speak, this was only in answer to scientific interrogation. During the First World War, a Polish scientist at the London School of Economics, Bronislaw Malinowski, spent two years on a small island in Melanesia and invented the modern form of ethnographic field enquiry. Malinowski insisted that the observer had to understand the native language and appreciate the actor's point of view in order to assess what he was told, to grasp the difference between what people said and what they did. Customs made sense in context, actions had meaning, people guessed at each other's intentions and adjusted their own behaviour accordingly in order to achieve goals which might be hidden, contradictory, culturally specific and situationally variable. The observer had to come off the verandah (as he said, in what became a famous phrase), because distance lent only the illusion of objectivity, and prevented genuine understanding of what was going on.

Ethnographic fieldwork on the Malinowski model has become one of the main research strategies in the human sciences, and its insistence on the context of action has influenced the study of other primates too, notably by way of the functionalist field studies of African apes initiated by Irvén DeVore in the 1950s. But notwithstanding its successes, it is evident that the move from the neutral, precise and systematic methods of the Torres Strait Expedition to the personal, messy and home-made methods of a Malinowski has its costs.

If the observer must participate in order to learn, then appeals to the authority of personal experience and to unique insights become uncomfortably prominent.



Obviously, it is not going to be easy to control for observer bias. So how can the reader judge between competitive accounts of a particular culture? These methodological questions troubled many anthropologists who did not take the substantive issues in the Freeman-Mead controversy very seriously. Freeman claimed that he could refute Mead by opposing his observations to hers, but this has turned out to be a difficult task.

To begin with, there have been other attempts to test Mead's findings, which have reached less severe conclusions. Lowell Holmes, an anthropologist who went to Mead's Samoan village 30 years after her study, specifically in order to reassess her work, concluded that her report was substantially accurate (*Quest for the Real Samoa: The Mead/Freeman Controversy and Beyond*; Bergin and Garvey, 1987). In the film, Freeman tries to show that Holmes is biased in favour of Mead for personal reasons, but does not challenge Holmes's findings in a systematic way. Other psychologists and anthropologists who have done research in Samoa are divided in their judgements of her work. (For examples of the various views see "Speaking in the Name of the Real", *American Anthropologist*, Vol. 85, 1983, and Holmes's review of relevant studies in Chapters 8 and 9 of his *Quest for the Real Samoa*.)

But even if Freeman's ethnography is reliable, this does not necessarily demonstrate that Mead got it wrong. She worked in another part of Samoa (under a different colonial regime, with different missionary influences and with a different economic base), and she was working 20 years — and a world war — before Freeman began his own field studies. It may be that both reported what they saw quite accurately, but that they saw different things because things were really different. Alternatively, they may have accurately reported the divergent perceptions of two different segments of the Samoan population. It is perfectly possible that Samoans had two conflicting theories about adolescence, one put about by adults, the other an unofficial, underground ideology of the young people themselves. Freeman may be presenting the point of view of respectable elders, while Mead (who participated mainly in the lives of the adolescent girls) may be generalizing from what was an informal sub-culture.

According to a body of opinion within anthropology, uncertainties of this kind are inevitable and cannot be resolved. If action, even perception, is culturally conditioned, then this applies not only to the subject of study but to the anthropologist as well. No observation is neutral; no observer can free him or herself from the constraints imposed by culture, status and life history. If observers do agree, then



In the field: Derek Freeman in Samoa, 1946.

this is probably because they share a bias. In his book *Orientalism* (Pantheon, 1978), Edward Said has argued that 'Orientalists' constructed an Oriental world to suit the purposes of the Occident.

The Freeman-Mead controversy has therefore fed a 'reflexive' tendency in ethnographic enquiry. The observer should be conscious of the contingent nature of research, and the final text, the ethnography itself, should not mimic scientific presentations but should be experimental, multi-vocal, ironical. Attention should be paid to the circumstances which produced the classic ethnographies, and to the rhetorical tricks which are used to persuade the reader of the ethnographer's authority. (See *Writing Culture* edited by James Clifford and George E. Marcus; University of California Press, 1986. And George E. Marcus and Michael M. J. Fischer's *Anthropology as Culture Critique: An Experimental Moment in the Human Sciences*; University of Chicago Press, 1986.)

This movement drew inspiration from contemporary critical philosophies, particularly those of Foucault and Derrida, and came to constitute a 'post-modernist' school within anthropology, as in other social sciences. One relevant development was an ethnographic sociology of science, which began from Thomas Kuhn's proposition that scientific paradigms had many of the characteristics of ideologies. Scientists were studied as though they were exotic tribesmen. The intrepid explorers discovered that the practice of laboratory research bears little

relation to the methodological accounts which figure in the textbooks, and which preface reports of findings in scientific papers. The conclusion was that scientific discoveries are constructed and established by social processes; that scientific consensus is a function of academic politics. (See Steve Woolgar's *Science: The Very Idea*; Routledge, 1988. And Bruno Latour's *Science in Action*; Open University Press, 1987.)

Post-modernists in anthropology are acutely alert to the importance of disciplinary politics, which play such a part in their theories. Accordingly, they organized effectively within the United States, where the entrenched cultural relativism provided fertile ground for their doctrines. Elsewhere their influence has been patchy at best. Even within American anthropology there has been a spirited and increasingly powerful counter-reaction. (See P. Steven Sangren, "Rhetoric and the Authority of Ethnography" *Current Anthropology* 29:3, 405-435; 1988.)

Yet even if it has only a brief vogue, the post-modernist challenge has already had a significant impact. Those who are formulating a response would not, on the whole, propose to reinstate the fiction of an objective observer who can bear witness to another culture 'as it really is'. Nor do they deny that research is a social process, and that ethnographies are social constructs. But this does not mean that there can be no firm standards. There is a discipline inherent in the process of ethnographic discovery. The observer and the subject do not simply circle round each other in mutual incomprehension — rather, they engage in a dialogue in which tentative agreement may be reached about actions and their meanings.

The ethnography which results is not a work of art, to be read more or less perceptively. It is a contribution to a debate about specific social processes and their significance. An ethnography will be taken up by a community of experts (including natives) who can call upon their experience of life in particular societies, and who will refer to a body of published observations and interpretations written from different perspectives and within a variety of disciplinary traditions. There is, then, an authority in ethnography, one which is not necessarily embodied in any one account, but which is emergent in the processes of expert research, comparison, evaluation and debate. Derek Freeman's crusading style does a disservice to rational academic discourse, but his dogged polemics have at least made many people aware that there are considerable issues at stake in contemporary anthropological research. □

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# Heliocentric tangents

SIR—The heliocentric hypothesis, so ably championed by Copernicus and Galileo<sup>1</sup>, is authoritatively said to have originated with Aristarchus of Samos in the third century BC. *Sand-Reckoner*, written by Aristarchus's younger contemporary Archimedes before 216 BC<sup>2</sup>, attributed to Aristarchus a book containing the hypotheses "that the fixed stars and the sun remain unmoved, [and] that the earth revolves about the sun in the circumference of a circle, the sun lying in the middle of the orbit . . .". Aristarchus's (lost) book is thought to have "clearly . . . also included some kind of geometrical proof"<sup>3</sup>.

Aristarchus had also produced a treatise *On the sizes and distances of the Sun and Moon*, which has survived intact. Its "excellent" methodology confirms that Aristarchus's (probably later) heliocentric hypothesis was similarly "not irresponsible" but rather the work of a "conscientious astronomer"<sup>4</sup>.

Nicholas Copernicus's *De Revolutionibus* (1543) acknowledged Aristarchus but not his heliocentric hypothesis<sup>5</sup>; however, it seems certain that Copernicus was also acquainted with the latter. For example, his original manuscript had referred to the opinion of Aristarchus on the movement of the Earth, but this reference was subsequently "suppressed"<sup>6</sup> or "scored out"<sup>7</sup>. Moreover, in relating the views of Philolaus, Heraclides and Ecphantus on the question of movement of the Earth, Copernicus's Preface quoted from *De Placitis Philosophorum* of pseudo-Plutarch, a work in which may also be found: "Aristarchus places the Sun among the fixed stars, and holds that the Earth moves around the Sun's circle"<sup>8</sup>. And *De Revolutionibus* (IV, 32) cites Archimedes' *Measurement of the Circle*, a treatise commonly found in the company of *Sand-Reckoner*<sup>9</sup>.

Copernicus's unquestionably pivotal contribution to astronomy was his grand revival of the heliocentric hypothesis as a systematic planetary theory<sup>1</sup>. But in order to fit theory to observations, Copernicus had retained the geometric devices used by Ptolemy (the deferent, epicycle and excentric), and had referred details of planetary movements not to the Sun but rather to the centre of the Earth's orbit. Because of technical and other difficulties with the copernican system, the astronomer Tycho Brahe (1546–1601) rejected it. Brahe had compiled an unrivalled set of observations, which he thought would demonstrate that the Sun and Moon travel around the Earth while the other planets travel round the Sun<sup>10</sup>.

After Brahe's death, Johannes Kepler (1571–1630) invested years in the analysis of Brahe's data, culminating in the derivation of Kepler's three laws of planetary

motion. These provided a precise and enduring mathematical characterization of the heliocentric hypothesis, thus serving to support the position of Copernicus while ironically refuting that of Brahe.

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## CFC photolysis

SIR—It is certainly possible (*Nature* **338**, 100; 1989) that "the great Antarctic ozone hole is a place where [CFCs and their products are] washed out on to the ice-cap". But this is not the rate-limiting factor in the very long tropospheric lifetimes of CFCs. The rate-limiting factor is simply the rate at which CFCs are carried up into the stratosphere to be photolysed. The sites of this photolysis are the middle and high stratosphere outside the polar night, not the ozone hole; and all the evidence points to the conclusion that this rate is controlled by atmospheric dynamics and that it is not particularly sensitive to details of how and where photolysed material returns to the troposphere. If anything, the throughput could be a little weaker in strong ozone-hole years.

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## Status of science

SIR—I agree with the gist of your leading article "Science squeezed" (*Nature* **338**, 1; 1989) on the shortage of young researchers in Britain. Research should be shown to be an honoured as well as an honourable profession, but the salaries paid by the state are surely indicative of the public's view of the comparative standing of the profession, as we have recently seen in the case of nurses.

I would like to take hospital physicists as an example of the standing of scientists. They have recently accepted, under duress, a pay rise of only 5.5 per cent, whereas the medical profession has been awarded a rise of more than 8 per cent. This is a fairly typical experience for scientists employed by the National Health Service and the story could probably be repeated in other

countries. I realize that hospital physicists are not predominantly engaged in research, but the country is tragically short of physicists in many areas. It is not surprising that there is now a substantial shortage of hospital physicists, yet the British public was only recently outraged when the calibration of an instrument used for radiotherapy in a hospital was wrongly set.

Lord Zuckerman, in *Scientific American* (September 1988, page 106), said that the most important element in the role of the scientific adviser to the chief executive (president or prime minister), is to "advise . . . on whether or not the country's educational institutions are turning out enough adequately trained manpower to fill the jobs that determine the well-being of the nation. A president, . . . has to feel confident that everything that can be done to satisfy this objective is being done, given the resources that can be made available." There is no evidence that this is happening in Britain.

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## Reprint research

SIR—Previous correspondents have argued for and against the reprint system, but have provided very little information about the pattern of reprint requests. You may therefore be interested in the sources of requests for reprints of my paper "Population dynamics of starlings in New Zealand" (*N.Z. J. Ecol.* **4**, 65–72; 1981). Starlings, being cosmopolitan, should interest all countries; publication yielded a nice collection of stamps and the following tally: United States 24, Canada 4, Finland 4, France 4, Japan 4, Hungary 3, Spain 3, Sweden 3, Australia 2, Belgium 2, Czechoslovakia 2, East Germany 2, West Germany 2, Israel 2, Poland 2, Alaska 1, Denmark 1, Italy 1, Latvia 1, Mexico 1, South Africa 1, Soviet Union 1, United Kingdom 1.

Given these data, one may speculate. Has the Soviet Union 24 times as many Xerox machines as the United States, or has the United States 24 times as many starlings? What does the United States do with all these reprints when it is said that Americans never quote overseas literature? If only one in 24 Soviets reads English, what do the English read? The intense rivalry between East and West Germany is mirrored in the exactly similar number of reprint requests, but why the balance between Latvia and Mexico? Finally, look at New Zealand: 3 million people, and none could afford the stamp.

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# Complicated problems not yet soluble

While physics may remain the art of representing difficult problems as simple, even the simplest complicated problems appear to have the practitioners bemused.

EVERYBODY knows by now that physics is merely sleight of hand. Very few problems of the real world are exactly soluble, and then only because it has been possible to simulate the real by a much simpler problem. The whole of kinetic theory is nothing more than an account of the motion of billiard balls, or elaborations thereof. The vibration of a turbine blade in a jet engine is described in terms of what is essentially the motion of a pendulum, as if it were just another example of simple harmonic motion.

That it may be far from simple to tell from first principles what are the frequencies of vibration of a turbine blade only serves to illustrate the point: the most widely used techniques are in at least two senses approximate — the predicted frequencies of vibration will be more or less in error and, even more significant, the only certainty is that any finite list of vibration frequencies must be incomplete.

This state of affairs is widely if not ostentatiously acknowledged. If challenged, people will readily turn the question against those who ask it, saying that the simulation of so many complicated by so few simple problems is a measure of the sophistication of their craft. But the business of chaos is a reminder that not everything is dealt with simply.

Even apparently simple dynamical systems capable of simple harmonic motion will behave unpredictably if, for example, there are forces which are not proportional to the displacement or some time-derivative of it. But while such a motion may be complicated, the same cannot be said of the underlying system, which has only two degrees of freedom (one space coordinate and time). That is the dark side of the claim that physics is the craft of making complicated problems simple: the search for simplicity blinded people to complexity.

The study of truly complicated systems is another matter, but the past few years have seen their surreptitious accumulation. These are the problems with many degrees of freedom in which the multiplicity is of the essence.

A simple regular idealized crystal lattice is a good illustration. If there are  $N$  atoms at the lattice points,  $3N$  coordinates will be needed to specify their displacements from their equilibrium positions and there will be  $3N$  frequencies of vibration which, once calculated, will describe the vibration of the crystal as if it were the vibration

of any one of  $3N$  oscillators.

But if the lattice points are occupied by quantum spins (as in, say, a ferromagnet), and if the problem is to calculate the response of the system to an external field, there is no such route to simplicity. The state of all spins at each instant is essential to an understanding of how the system as a whole behaves. Ways of approximating to an understanding by what are called, for example, 'mean-field' approximations, may yield gross measures of, say, magnetization — but then only well away from the Curie temperature. Subtle features of such a system, say the speed of motion of the boundaries between magnetized-domains, for example, remain incalculable.

The recently accumulated examples of this kind keep many people awake at night. Neural networks are a fashionable example, where the instantaneous state of all the neurons in an interconnected network is crucial to an understanding of how one state of the network switches to another. But that is merely one reason for remarking that, against the prevailing grain of physics, there appears from the literature to be a small group of people working away at systems which, by having many degrees of freedom, are inherently complicated, but which are nevertheless tractable.

There is, for example, the case of the proposed use of arrays of interconnected superconducting Josephson junctions as means of analysing the frequency of millimetre-wave signals. Kurt Wiesenfeld and Peter Hadley from the Georgia Institute of Technology have now given a fascinating account of this system (*Phys. Rev. Lett.* **62**, 1335; 1989). Those interested will find an earlier preliminary account (with M.R. Beasley) helpful in fixing ideas, as the saying goes (*Appl. Phys. Lett.* **52**, 1619; 1988).

A Josephson junction is a two-dimensional superconducting circuit (on a chip) whose dimensions are such that the wavefunctions of the circulating electrons have dimensions comparable with those of the whole device. One consequence is that the electrical behaviour of a single junction in an array is determined by the phase difference between its electronic wavefunction and that of neighbouring wavefunction. Josephson junctions, in other words, are a means of making quantum properties macroscopically measurable. Arrays can be fabricated with a difficulty that may

seem child's play to those who make fortunes in the semiconductor industry.

But to make such arrays function as advertised in the millimetre-wave business, all the junctions in an array must oscillate together, in phase with one another. The difficulty is that the equation that determines the oscillation of a single junction in an array is inherently non-linear. (Apart from a few constants, the quantity representing the restoring force in simple harmonic motion appears as the trigonometric sine of that quantity, and there is no presumption that the equivalent of the restoring force is small, because the argument of the trigonometric sine can be anywhere between 0 and  $2\pi$ .) The consequence is the sequence of *ad hoc* arguments by which the authors reach their interesting conclusion.

This is how it goes. It is easy enough to show that the condition in which all the junctions in an array oscillate as one, with no phase differences between them, is a solution of the equations; that is why arrays of Josephson junctions were advocated as local oscillators in the first place. The practical question is whether such an oscillating state will be stable. Bereft of more explicit alternatives, the authors go through a counting exercise, adding up the number of oscillatory states of a Josephson array in which the phases will be out of synch.

The obvious practical question is whether the discriminatory power of a local oscillator built on lines like this will increase as the number of junctions in the array increases. Naturally, there must be a tendency in that direction, but there is also a countervailing influence. Put crudely, each out-of-synch state of oscillation is potentially a state into which the intended stable state may be kicked by random noise — and the out-of-synch states are crowded ever more closely together (in what is known as phase space) as the number of elements increases. A further consequence, demonstrated only by numerical simulation, is that an increase in the number of elements in an array quickly decreases (virtually to zero) the time an array will spend in any particular oscillatory state.

On the face of things, that would seem to spell the end of Josephson arrays as millimetre-wave detectors, but really it is merely a signal that much more needs to be learned of a complicated simple system.

John Maddox



# Defective viruses to blame?

Robin A. Weiss

THREE new papers on an acquired immune deficiency syndrome in mice caused by a strain of murine leukaemia virus suggest that a defective genome is responsible for the immunodeficiency. Janet Hartley, Sandy Morse and colleagues<sup>1</sup> at the National Institutes of Health have found an unusual pattern of genetic susceptibility to murine AIDS consistent with a defective genome being carried by different replication-competent 'helper' viruses. This has been followed up by the molecular identification, also by Hartley and colleagues<sup>2</sup>, of the defective virus. And an apparently identical genome has been independently identified by Paul Jolicoeur and colleagues in Montreal, as they report on page 505 of this issue<sup>3</sup>. After cloning of the defective virus, which retains *gag* sequences in its 4.8-kilobase genome, and rescue by non-pathogenic helper viruses, inoculation into mice leads to the induction of AIDS<sup>2,3</sup>. These findings supplement that from Jim Mullins's group<sup>4</sup> which shows that in cats, immunodeficiency can be caused by a defective feline leukaemia virus.

Many classes of animal RNA virus accumulate defective variants when propagated serially at high multiplicities of infection. This was first observed by von Magnus with influenza virus<sup>5</sup>. Such defective viruses frequently interfere with the replication of full-length, non-defective genomes and they may play a role in pathogenesis and persistent infection<sup>6</sup>. Defective retroviruses, however, do not typically interfere with their helper viruses and are not simply truncated forms of the competent virus; they contain recombinant viral or cellular sequences that can directly influence the pathogenesis of the virus population.

## Retroviral oncogenes

The notion that defective variants of retroviruses cause specific disease was raised more than 25 years ago, when Hanafusa *et al.*<sup>7</sup> showed that the Bryan strain of Rous sarcoma virus was defective. This classical study led eventually to the identification of oncogenes derived from cellular genes, usually by insertion at the expense of viral genes, thereby rendering the viral genome replication-defective. Nearly all retroviral oncogenes are carried in defective viruses requiring helper viruses for transmission.

That defective retroviruses can carry 'scrambled' viral genes determining new disease patterns, rather than cellular genes, was first seen with the spleen-focus-forming virus (SFFV) component of Friend erythroid leukaemia virus. The defective SFFV genome encodes an incomplete

retroviral *env* gene derived from exogenous, 'ecotropic' and endogenous, 'xenotropic' sequences. This new form of glycoprotein probably interacts with specific 'dual-tropic' receptors on erythroid precursor cells to trigger their proliferation<sup>8</sup>. As the term 'viral oncogene' is usually restricted to gene sequences of mainly cellular origin, and of course pertains to those genes inducing neoplastic transformation, we need a new term for retroviral genes of viral origin specifying particular disease forms — I would offer as a suggestion 'pathogenes' with path as the generic equivalent to onc.

The SFFV gene determining erythroid leukaemia is derived from *env* sequences<sup>8</sup>, as is the gene inducing feline AIDS in defective feline leukaemia virus<sup>4</sup>. A recent paper by Poss *et al.*<sup>9</sup> indicates that the property of the *env* protein of the defective feline AIDS virus which makes it cytopathic for T cells lies in its post-translational glycosylation pattern. With murine AIDS, however, it appears that the functional path gene in the defective virus is derived from the *gag* gene. Aziz, Hanna and Jolicoeur<sup>3</sup> have sequenced the 4.8-kilobase genome and found four open reading frames, two corresponding to portions of *gag*, one to part of *pol* and one to part of *pol-env*.

The p12 region of the largest open reading frame (586 amino acids) has only 40–50-per-cent identity with Gag p12 of other murine retroviruses, and it is this product that the authors suspect is responsible for inducing AIDS. Chattopadhyay *et al.*<sup>2</sup> also implicate a *gag*-related product because Gag antisera including anti-p12, but not anti-Pol and anti-Env sera, immunoprecipitate a 60-kilodalton protein from a murine cell line carrying the defective genome in the absence of helper virus.

The study of defective murine and feline leukaemia viruses causing immune deficiency syndromes should enhance our understanding of the various ways retroviruses induce disease. Being oncoviruses rather than lentiviruses and therefore less closely related to the human immunodeficiency virus (HIV), their use in screening anti-viral drugs is doubtful; if one wishes to use an oncovirus for this purpose, Friend virus induces disease in mice more quickly with a more quantifiable endpoint.

Aziz *et al.*<sup>3</sup> cite striking similarities of murine AIDS to human AIDS, though there are striking differences too. The disease was first described as lymphoma<sup>10</sup>, owing to what is now believed to be a polyclonal B-cell hyperplasia rapidly induced by the virus. A detailed study<sup>11</sup> of

the syndrome showed that a profound suppression of humoral and cellular immunity soon follows the lymphoproliferative phase. Both the B-cell hyperplasia and the immune suppression seem to be T-cell dependent, because the virus affects nude mice much less severely<sup>12</sup>. However, it is not clear that the virus infects T-helper cells, and the macrophage reservoir and neurotropism characteristic of HIV have not been examined in murine AIDS. In my view, the diseases induced by HIV-related, T-lymphotropic lentiviruses of cats (FIV), cattle (BIV) and monkeys (SIV) will prove to be more proximate models of human AIDS.

## Role in human AIDS

Nonetheless, the discovery of defective oncoviruses inducing severe immunosuppression in cats and mice raises the question whether defective HIV genomes might play a role in human AIDS. Replication-competent, molecularly cloned HIV and SIV genomes have not yet been shown to induce AIDS, and even if they do, that might require the generation of defective, variant genomes during the long incubation period between infection and manifestations of disease.

Several HIV laboratories are finding a myriad of viral forms when sequences are amplified directly from infected tissues, rather than selecting for replication-competent viruses in cell culture. Simon Wain-Hobson (personal communication) refers to such HIV populations as 'quasi-species' because they represent greater diversity than might be expected from classical population variability. The mixture of defective oncoviruses and the helper viruses required for their generation and transmission represents a special case of quasi-species genome dynamics. No doubt defective forms of HIV will be carefully scrutinized at the molecular level. □

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# Global perspectives of chaos

Christopher H. Scholz

RECENT developments in nonlinear dynamics have not failed to capture the imagination of geophysicists, whose domain encompasses a great many nonlinear processes. For solid-Earth geophysicists, the most exciting possible applications are to mantle and core convection and, especially, earthquakes. Any segment of the stressed boundary between two plates exhibits a seismic cycle defined by the recurrence of large earthquakes, between which only minor seismic activity occurs. In many cases, this cycle does not seem to be periodic, but the question "is the system chaotic, and if so, in what way?" is yet to be resolved.

Condensed matter physicists and geophysicists came together at a recent meeting\* to discuss such questions. Each group is still learning the other's discipline, so that what transpired was a preliminary dialogue which nonetheless contains a foretaste of things to come.

The elastic rebound theory of earthquakes, developed by H. F. Reid at the turn of the century, is often inferred to predict the periodic recurrence of large earthquakes, but even in Reid's day it was recognized that the spatial heterogeneity of faults would result in aperiodic behaviour (G. K. Gilbert, *Science* **29**, 121-138; 1909). The seismic cycle can be thought of as a friction-damped oscillator with many spatial degrees of freedom, which is almost a prescription for chaos. As symptoms of this, it is already known that the system abounds in fractal distributions. The Gutenberg-Richter power-law size distribution of earthquakes, as near to universal as any geophysical observation, is one such fractal distribution, and it has also been found that the size distribution of faults, fault topography and even the grain-size distribution of fault gouges are fractal (D. Turcotte, Cornell University; C. Sammis, University of Southern California). Earthquake faults thus have the same fractal nature as other fractures, as discussed in a recent News and Views article by Robert Cahn (*Nature* **338**, 201; 1989).

The seismotectonic system can be regarded as a nested hierarchy of fractals, ranging from topography produced by active faulting down to the material

ground between the fault walls. Although they have learned these geometric facts, geophysicists have only just started to think about the dynamic systems that can give rise to such structures. Any models developed in other fields of nonlinear science, such as those employing cellular automata or renormalization groups (discrete-variable approximations of differential equations), are ripe to be imported to explain the physics of earthquakes.

Although most nonlinear studies to date have been of low-dimensional systems, a very general model of spatially extended dissipative systems has recently

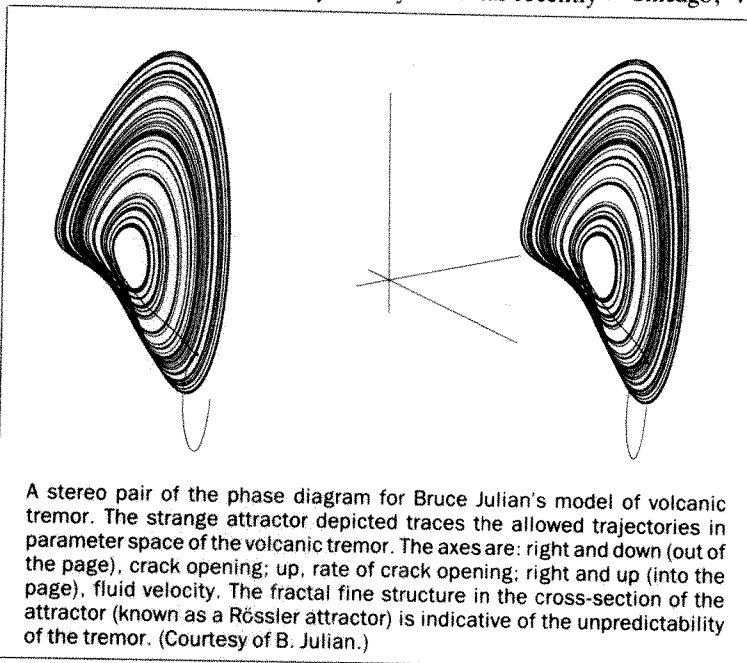
contrast, another cellular-automata model employing simple triggering criteria (T. Hirata, Tsukuba University) has the property that even if started randomly it becomes organized by phase-locking to produce regular large events. Earthquakes may have both of these characteristics. Large earthquakes must roughen the stress distribution on the fault, but in the intervening period, many small earthquakes must smooth it again to set the stage for the next large event.

Whereas the periodicity of the earthquake cycle is a central question for long-term earthquake prediction, short-term prediction depends on identifying the nucleation phase. Diffusion-limited-aggregation and viscous fingering models of nucleation (T. Halsey, University of Chicago; W. Klein, Boston University)

are instructive but not very applicable, as their finely branched structures are unlike earthquake rupturing, which tends to fill the plane. The several forms of a renormalization group model of bundles of parallel fibres under tension produce a chain-reaction nucleation (S. Solla, AT&T Bell Laboratories; W. Newmann, University of California, Los Angeles), but have the unfortunate property of predicting zero strength in the limit of an infinite bundle. This is because the entire bundle fails when the weakest fibre does, owing to the strong nearest-neighbour reloading.

Ropemakers long ago learned to avoid this property by braiding the rope,

which causes friction between strands to be induced by tension on the rope; this causes the problem to become three-dimensional because a broken fibre does not globally lose its load-carrying capacity. Earthquakes, being shear cracks, dissipate energy by friction over their entire surface and so will similarly have a less abrupt nucleation. Nucleation models based on rock friction constitutive laws exhibit scaling that depends on a critical slip distance (J. Dieterich, US Geological Survey, Menlo Park). The critical slip distance is a material (surface) property, which in uniform block-slider models determines the size of the slipping patch at instability (see my paper in *Nature* **336**, 761-763; 1988). However, in spatially extended models in the stable mode, chaotic motion can occur with this friction law, even in the case of spatially uniform parameters (F. Horowitz, Georgia Technical College). This may avoid a problem that seemed implicit in the model: the prediction of a minimum earthquake size.



been developed which predicts some of the characteristics of earthquakes (P. Bak, C. Tang & K. Wiesenfeld, *Phys. Rev. A* **38**, 364-374, 1988). This cellular-automata model has the property that it evolves to a self-organized critical state that is characterized spatially by a power-law size distribution of clusters and temporally by  $1/f$  noise ( $f$  is frequency). A simple visualization of it is of a pile of sand, in which the self-organized critical state is the maximum angle of repose, the size distribution of landslides is a power-law, and the sand flow off the pile is  $1/f$  noise. Earthquakes do have this size distribution, and the temporal signature can be checked by the study of earthquake catalogues.

Building further on this development is a model of a network of stick-slipping friction masses connected by springs (J. Langer, University of California, Santa Barbara). This model has the important property that even if it is started in a homogenous state it evolves to chaos. In

\* Earthquakes: chaotic or deterministic? Asilomar, California, 12-15 February 1989.

The origin of the fractal topography of faults was addressed by H. Herrmann (Saclay). In a square-grid model subjected to shear forces, crack growth occurs with pervasive branching, resulting in a fractally irregular crack traversing the grid with many side branches. In contrast, the tensile case produced a much more regular structure, with only minor secondary cracks. This result, not likely to be sensitive to the details of the model, explains the much greater complexity of shear to tensile cracks that is well known from both the field and laboratory.

In two other papers not related to earthquakes, modelling predicted chaotic behaviour for diverse phenomena. Volcanic (harmonic) tremor is a nearly continuous vibration that accompanies volcanic eruptions. Treating the source of the vibrations as magma flowing through a compliant crack, B. Julian (US Geological Survey, Menlo Park; see figure) finds that including radiative dissipation introduces a transition to chaos by 'period doubling': as the magma velocity approaches a critical

value, sub-harmonics of the vibration set in until the vibration becomes completely chaotic. And the onset of chaos by way of 'Hopf' and 'pitchfork' bifurcations, splitting of fluid trajectories, has been predicted in models of mantle convection (C. Stewart, Cornell University).

There are sophisticated methods for extracting from a time series of a chaotic process the structure of the 'attractor' (which defines the set of possible states), after which one has a better chance at guessing the system that produced the attractor (J. Sidorowich, University of California, Santa Cruz). But the question for earthquakes is whether geophysical data sets are adequate for this approach. Seismicity catalogues are generally much shorter than the recurrence time of large earthquakes, and it is not at all clear that the attractor for the loading cycle can be obtained from them. □

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can capture the available neurotransmitter and thus more pores can be opened (the postsynaptic mechanism). An increase in transmitter release might also occur by having each vesicle contain a greater quantity of neurotransmitter. On the postsynaptic side, an increase in strength could also result if each pore remained open for a longer time or if each receptor were to bind its ligand more avidly.

The question, then, has been whether LTP arises from a presynaptic mechanism (more transmitter release) or a postsynaptic mechanism (greater sensitivity of the target membrane). The second alternative, the postsynaptic mechanism, is easiest to believe because it is well established that the crucial signal for triggering LTP (a postsynaptic influx of calcium ions) occurs only in the postsynaptic neuron. Nevertheless, until recently, the weight of evidence favoured the presynaptic mechanism and one was forced to suppose that the triggering signal produced another message that was relayed from the target neuron back to the axon terminal. What Davis *et al.* are proposing is even more complicated.

One good way of detecting a postsynaptic mechanism is to apply neurotransmitter locally to the target neuron and see if it is in fact more sensitive after heavy synaptic use than before. If synaptic strength correlates with postsynaptic sensitivity to neurotransmitter, determined by direct glutamate application, then the mechanism is probably at least partly postsynaptic. If, on the other hand, the postsynaptic membrane is not more sensitive to exogenously applied neurotransmitter, either LTP arises through an increase in transmitter release, or the experiment is inconclusive because the right place on the postsynaptic neuron was not tested.

What Davies *et al.* find is that, even though LTP develops promptly after heavy synaptic use, postsynaptic sensitivity to exogenously applied neurotransmitter (more correctly, to a neurotransmitter analogue) develops slowly over about half an hour. The conclusion, then, is that the mechanism is presynaptic for the first half-hour and that gradually the postsynaptic membrane becomes more sensitive — some signal must pass from the postsynaptic membrane to the axon terminal to produce a transient increase in release. With

time, however, an increased sensitivity of the postsynaptic membrane grows so that LTP is maintained by a postsynaptic mechanism.

Although this mechanism seems complicated, it does fit with other recent observations. Kauer *et al.*<sup>2</sup> were the first to define clearly two temporal phases of LTP — they discovered a first, transient LTP component that lasts about half an

## LONG-TERM POTENTIATION

# Strengthening the synapses

Charles F. Stevens

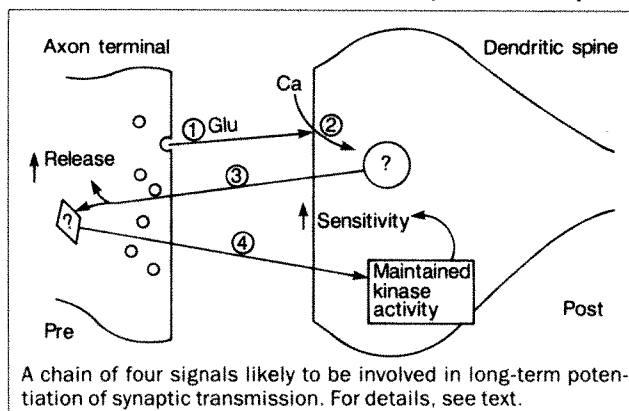
ON page 500 of this issue<sup>1</sup>, Davies *et al.* provide an answer to one of the important open questions about how long-term potentiation (LTP) works. LTP is a very long lasting increase in synaptic strength that is produced at certain synapses when they are used repeatedly — a sort of microscopic 'practice makes perfect'. This phenomenon has attracted much attention recently because it is thought to be the cellular basis for certain types of memory.

Whether the site of increased connection strength is presynaptic or postsynaptic — that is, whether it resides in the sending or the receiving neuron, has been a controversy for some time. The answer Davies *et al.* give is more complicated than most had hoped for: it is both. For the first half-hour after a synapse has been heavily used, the increased strength arises in the presynaptic terminal, and then the processes causing LTP develop in the postsynaptic membrane.

Just how can a synapse get stronger? Understanding the possibilities requires a brief review of how synapses function. Synaptic transmission works by the nerve terminal (the presynaptic element) releasing neurotransmitter through an exocytotic process. In the synapses where LTP has been demonstrated, this is glutamate, which is contained in 40-nm vesicles that are released into the cleft between the pre- and the postsynaptic

cells; the glutamate diffuses rapidly across the cleft and binds to several distinct types of glutamate receptors in the membrane of the target neuron (the postsynaptic element). These receptors open pores through which ions flow. Synaptic strength, then, is determined by the quantity of ions that flow through open pores into the postsynaptic neuron.

A synapse will be stronger if more neurotransmitter is released by the axon



terminal or if the postsynaptic neuron responds more vigorously to whatever quantity of transmitter is present, or both. The two possibilities that are usually considered are: first, that the axon terminal releases more vesicles, each containing a fixed quantity of neurotransmitter (the presynaptic mechanism for strengthening); or second, that the target postsynaptic membrane contains more receptors than



## GEL ELECTROPHORESIS

## DNA molecules observed

Ted Richards

hour, the same duration as the presynaptic phase of LTP now defined by Davies *et al.*. This transient component can be evoked in isolation by applying neurotransmitter directly to synapses without stimulating the axon terminals<sup>2</sup>.

More recently, Malinow *et al.*<sup>3</sup> have discovered a second way to define transient and maintained phases of LTP. A persistent speculation has been that one fundamental mechanism in the storage of memories is the covalent modification of some (unidentified) proteins by kinases. During an investigation of this hypothesis, Malinow *et al.* found that when kinases are inhibited, the transient phase of LTP is still evoked, but that the maintained phase fails to develop. According to the new results of Davies *et al.*, the transient phase of LTP would be presynaptic, and the maintained component would result post-synaptically from an increased sensitivity of receptors to neurotransmitter resulting from some kinase activity.

All this work has many potential sources for artefacts. If the results are taken at face value, though, the following picture of four inter-related signals emerges (see figure). The first is glutamate that is released from the axon terminal and acts on the postsynaptic membrane to change the postsynaptic voltage and — under the right circumstances — permits an influx of calcium ions. The resultant change in postsynaptic calcium concentration constitutes the second signal and produces, by an unknown mechanism, the third signal that is sent from the postsynaptic cell to the axon terminal where it increases the quantity of glutamate released for each nerve impulse that arrives.

This third signal, in conjunction with some trace of recent heavy use of the axon terminal (calcium again?), causes the fourth signal to be passed from the axon terminal back to the postsynaptic target neuron where it somehow produces a maintained kinase activity that, in turn, results in an increased postsynaptic sensitivity to glutamate.

Why is such a complicated scheme needed? One reason may be that the brain wants to be very careful to store only valid memories, and therefore needs to be sure that parts of the system are not activated accidentally. By having this 'handshaking' scheme, checks are instituted to ensure that the necessary conditions are met for having an appropriate increase in synaptic strength. Whatever the reason, there is clearly still much to learn about the mechanisms involved. □

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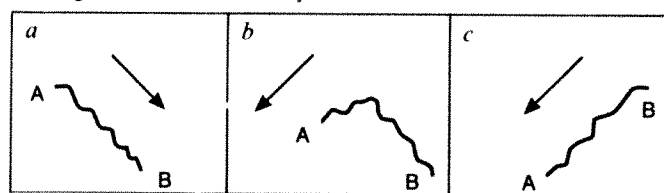
It should be obvious to any reader of *Nature* that gel electrophoresis of DNA has revolutionized molecular biology. It is therefore odd that the precise mechanism whereby DNA molecules, moving under the influence of an electric field in a gel, are separated according to their length, is but poorly understood. The standard description, based on the reptation model of De Gennes, has the molecule snaking down a 'tube' among the gel fibres with the head moving in a direction biased in the direction of the field. This model accounts for the observed inverse relation between mobility and molecular size, which breaks down for long molecules or

toy which consists of a squat helical spring that 'walks' down stairs.

The picture is broadly similar at low-strength fields, but here coils may also start to form in the middle of the extended chain; these may work their way to the trailing end of the chain as the front moves down the field. When they reach the end the trailing end may take off downwards, so forming an inverted 'U' with the chain looped over several obstructions. Eventually the longer end pulls the chain free.

Smith *et al.*<sup>3</sup> and Schwartz and Koval<sup>2</sup> describe experiments in which the motions of real DNA molecules are directly observed. The idea is novel but

Movement of a DNA molecule in an electric field. *a*, The molecule is aligned along the field with end B leading. *b*, The field is reversed and the chain is starting to align in the new direction. The centre of mass has not moved downwards. *c*, The molecule is now aligned in the new direction with end A leading. It can now start to move.



at high electric fields when the mobility is found to be independent of size. It now seems, however, that this model is oversimplified. Deutsch and Madden<sup>1</sup> have now described a computer simulation of the movement, a study which is complemented by experimental observations of real DNA molecules by Schwartz and Koval on page 520 of this issue<sup>2</sup> and by Smith *et al.* in a paper recently published in *Science*<sup>3</sup>.

Deutsch and Madden model the DNA molecule by a chain of charged beads and the gel matrix by a rectangular array of obstructions. They set up equations which determine the motion of the beads and solve these numerically on a computer. In this way they obtain a series of 'snapshots' which portray the conformations of typical chains as time progresses. The results unexpectedly show that a chain alternates between a coiled conformation and one in which the chain is extended in the direction of the field. Exactly what happens depends on the field strength.

Starting at the stretched-out phase, Deutsch and Madden find for high-strength fields that the leading end starts to coil up, eating up the rest of the chain as it moves down; this coil becomes entangled with the gel obstructions. Then one end proceeds to move down leaving the other looped over the gel fibres. As this leading end moves in the direction of the field, the loops slip off the obstructions, until eventually the trailing end is freed and the cycle starts again. The whole cyclic motion is reminiscent, to those who have seen it, of the motion of a 'slinky', a

simple. The DNA molecules are first labelled with a fluorescent dye and then allowed to migrate in an electric field in a thin layer of agarose on a microscope slide. When the specimen is illuminated with light of a suitable wavelength, the fluorescing groups reveal the conformation of the DNA chain. The image of the wriggling chain can be recorded on video tape. The motions so recorded by both groups are in good qualitative agreement with the simulations obtained by Deutsch and Madden, and at some variance with standard reptation model.

Deutsch and Madden<sup>1</sup> discuss the assumptions inherent in the reptation model which appear to be violated in their simulations; they also show that the mobility of long chains in high electric fields is independent of the chain length, in agreement with both standard reptation theory and experiment. This effect leads to an experimental impasse whereby any DNA chain longer than about 20 kilobases cannot be resolved using conventional methods. This impasse is much to the chagrin of molecular biologists, who wish to resolve ever larger pieces of DNA, up to the ultimate goal of separating whole eukaryotic chromosomal DNA.

Several groups have found ways out of this impasse by periodically altering the direction of the field. In this way it is now possible to resolve molecules the size of the yeast chromosome, about 2 megabases. Several such methods involve fields oriented at an obtuse angle to each other so that molecules traverse a zig-zag path along the bisector of this angle. The

reptation model provides a schematic picture of how this works (see figure): at each change in field orientation, both ends of the chain move off in the new direction. Only after a certain time, dependent on the length of the chain, is a molecule completely oriented in the new direction; this process must be completed before further net progress is made. Thus longer molecules are retarded more than shorter ones. This mechanism does not explain all the experimental results, particularly those in which the field is periodically reversed.

The simulations of Deutsch and Madden contain some artificial features: they are conducted in two rather than three dimensions (which may not be serious), and the rectangular array of

obstructions cannot correctly mirror the true structure of the gel, which is largely unknown. Further work should ameliorate these difficulties and also suggest what happens when the direction of the field is changed. Nevertheless, it is hoped that these insights and observations will stimulate experimentalists to devise methods of resolving longer and longer DNA chains. □

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## CRYSTALLIZATION

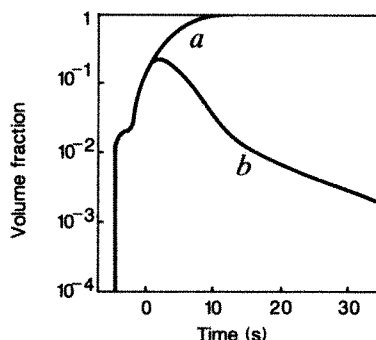
# Views of interfacial ephemera

A. Lindsay Greer

QUANTITATIVE measurement of crystallization kinetics in alloy systems has become possible in recent years through the annealing of metallic glasses. In the glasses the atomic mobility is sufficiently low for the rates of crystal nucleation and growth to be measurable and for the sample temperature to remain uniform during the reaction. These are conditions which do not apply in the more common case of crystallization from a liquid. Newly reported work by Sutton *et al.*<sup>1</sup> shows that important information on the structural changes during crystallization of a metallic glass may be lost if the experiment is not conducted in real time during the anneal. The transient interfacial phases suggested in the new work merit further study because of their potential significance in understanding crystallization kinetics not only in glasses, but also in liquids.

In conventional structural studies of the crystallization of metallic glasses, X-ray diffraction experiments are performed at room temperature after annealing. In contrast, Sutton *et al.*<sup>1</sup> use synchrotron radiation to study the transformation *in situ*, with time resolution of 3 ms. The study is of the formation of the body-centred tetragonal phase of NiZr<sub>2</sub> from metallic glass of the same composition. Diffraction patterns from the partially transformed samples cannot be reproduced by superposing the patterns from the initial amorphous phase and from the final NiZr<sub>2</sub>, so that the authors postulate there is a precursor phase. It has broad diffraction maxima and is a transient structure existing at the interface between the growing-crystal phase and the amorphous phase. As shown in the figure, the phase is most noticeable in the early stages of the transformation. It is found only during anneals at higher temperature when the transformation is more rapid.

That metallurgical transformations can proceed through intermediate stages (rationalized, for example, by Turnbull's step-entropy rule<sup>2</sup>) is well appreciated, and is exploited in the conventional practice of quenching and annealing, as in the production of precipitation-hardened alloys. The annealing is stopped before



The time dependence of the total crystalline volume fraction (a) and the volume fraction of intermediate crystalline phase (b) for an anneal of NiZr<sub>2</sub> metallic glass at 680 K (redrawn from ref. 1).

the final equilibrium is reached, to preserve the intermediate microstructure which is technologically useful.

But the possibility of an intermediate phase that exists only during the reaction and that cannot be preserved by cooling is not commonly considered. Such a concept is related to the activated-complex or transition state used in considering the mechanisms and rates of chemical reactions. For crystallization, a first-order transformation, any intermediate state must be associated with the interface between the two phases. One instance where a transient interfacial phase has previously been postulated<sup>3</sup> is the explosive crystallization of thin films of an amorphous semiconductor, such as germanium, which is explicable only if

there is a thin layer of metallic liquid, maintained by the release of latent heat, at the interface between the solid amorphous and crystalline phases, both of which have covalent tetrahedral bonding.

The nature and origin of the possible transient phase in the crystallization of Ni–Zr glass must be very different, but likewise it may be significant in analysing crystal growth. The attachment of atoms to a crystal growing in a liquid or amorphous phase may involve diffusive jumps, leading to a maximum, diffusion-limited growth velocity of around 10 m s<sup>-1</sup>. But measurements of dendrite velocities in undercooled melts led Turnbull<sup>2</sup> to suggest that for pure metals, the crystal growth rate may be limited only by the collision rate of atoms with the interface. The collision-limited growth rate would then be near the speed of sound, about 200 times the diffusive speed. From pulsed laser experiments, there is firm evidence for each type of growth; collision-limited for pure metals<sup>4</sup>, but diffusion-limited for intermetallic compounds<sup>5</sup>. The transition from one type of growth to the other as the solute content is increased has not yet been satisfactorily analysed.

Sutton *et al.*<sup>1</sup> suggest that the transient phase is a poorly ordered version of the final crystalline structure. Thus there is evidence for growth proceeding in two stages, crystallization followed by chemical ordering, in a manner consistent with the step-entropy rule. Diffusion-limited growth may arise when there is a need for rearrangement of the atomic short-range order at the interface, and just such a process may be revealed by the *in situ* study. The transition between types of growth may be elucidated by studies of the same type on growth of extended elemental solid solutions and of intermetallic compounds with different structures.

The greater fraction of intermediate phase detected at higher temperatures could reflect, as the authors suggest, the higher nucleation frequencies and greater interfacial areas during the transformation. On the other hand, it is possible that it could be associated with the ordering lagging further behind the faster interface. A transition from growth of an ordered phase to growth of a disordered intermediate could affect not only the growth rate, but also the degree of faceting of the crystal through the well-known link with entropy change across the interface<sup>6</sup>. □

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## EPSTEIN-BARR VIRUS

# Chance and felicity

John Galloway

TWENTY-five years ago this week the first report appeared of what came to be known as Epstein-Barr virus (EBV)<sup>1</sup>. The finding of a previously unknown virus in tumour cells cultured from a patient with (Burkitt's) lymphoma was a signal event for the Cancer Research Campaign (CRC), then the British Empire Cancer Campaign, and one that the campaign is celebrating next week with an international conference\*.

The progress of science is littered with happy coincidences, chance observations and meetings that mark the beginning of successful lines of research. But few have involved as much serendipity in their early stages as EBV. The story began in the 1950s when Denis Burkitt noticed a child in the African teaching hospital where he worked with swelling in all four quadrants of the jaw. It seemed to be neither a tumour nor an infection, but three weeks later he saw exactly the same thing again in another patient. No longer a curiosity, the condition became a medical problem, and in time it was realized to be the commonest childhood cancer in Africa — and indeed that other childhood lymphomas were simply different manifestations of the disease — now called Burkitt's lymphoma.

Burkitt noticed that the disease had a characteristic geographical distribution, clearly related to climate. Climate sugges-

ted biological transmission, possibly an insect vector. He turned out to be right about the climatic connection — his lymphoma had exactly the same geographical distribution as malaria — but not about the insect vector. His work attracted little interest until he gave a lecture in 1961 which was attended by Tony Epstein.



Epstein, Barr and Achong in 1964 (courtesy of M. A. Epstein).

Epstein, persuaded by Burkitt's argument for a biological cause, decided it must be an oncogenic virus<sup>2</sup>. Viruses were known to cause and spread cancers in animals, and, although there was really no evidence, were thought to cause human cancers.

At this point the Campaign paid for Epstein to visit Burkitt in Kampala to obtain biopsy material. In a cell line established in culture from a tumour biopsy, they found, using the electron microscope, a new, large, icosahedral herpes-like virus in the tumour cells. The finding was reported in *The Lancet* and was later named after Epstein and his PhD student Yvonne Barr who, with Bert Achong, was

his co-author. It was fortunate that the laboratory had available thin-section electron microscopy; the virus could not have been detected otherwise as it was inactive in all the biological assays then available.

The serendipity which marked the early work on EBV was repeated later in the Henles' laboratory in Philadelphia, where a chance event revealed that EBV was also the cause of infectious mononucleosis — glandular fever. A technician whose blood was being used as a negative control in serum-antibody tests for EBV infection contracted glandular fever while on holiday and developed a high concentration of antibodies against the virus. A sero-epidemiological prospective study then showed conclusively that EBV was indeed the cause of the disease.

So much for history. What do we know about the virus? Does it really contribute to Burkitt's lymphoma — and nasopharyngeal cancer — as is claimed? Is there any point in developing a vaccine against EBV to prevent these diseases? Why else should EBV be the subject of so much and such intense research?

EBV is a typical herpesvirus, the exemplar of the  $\gamma$ -herpesvirinae. It contains 170 kb of double-stranded DNA, encoding something like 100 proteins, and its genome structure is known in great detail<sup>3</sup>. It is noteworthy for its complexity and remarkable examples of long range splicing. The large number of proteins it encodes seems to be related to its need to cope with many types of cellular metabolism — it grows in B cells and epithelial

\*Epstein-Barr Virus: The First 25 Years, Oxford, 9-13 April 1989.

## Mummy deterioration halted by nitrogen atmosphere

MANY museum objects are damaged by microorganisms and insects (see A. David *et al. Science in Egyptology*; Manchester University Press, 1986). We have developed a method to control biological damage and to provide optimum display conditions, and have used a 3,000-year-old Egyptian mummy as a model (see figure). This woman was 40 years old when she died and came from a rich family, shown by the mummification treatment and by a layer of gold over her eyes. The mummy was placed in a hermetically sealed display case and the air replaced by nitrogen at low relative humidity. The combination of low relative humidity (35-40 per cent) and oxygen level (less than 2 per cent) significantly decreases



the biological activity (95 per cent) of microorganisms isolated from the mummy, assessed using carbon-14 tracers. A sealed display case with controlled humidity and a nitrogen atmosphere was designed at the Getty Conservation Institute by Frank Preusser. This is a prototype for showcases to be used in the Egyptian Museum in Cairo. By contrast with common fumigation systems, nitrogen is safe, inexpensive and easily handled. In art object preservation, a nitrogen atmosphere could be an effective means for drying and disinfecting water-damaged materials, and for eliminating insects from museum collections.

Nieves Valentin

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cells and can undergo a latent or lytic infection. Since the number of its potential hosts is small (humans and a few primates), its characteristic property of persistence for long periods of time in a latent stage from which it can be rapidly activated is sensible behaviour.

The virus is very common, more than 90 per cent of the world's population is infected. It is ironic that a suggestion of a viral involvement in a not very common cancer, of limited geographical distribution, should have led to the discovery of one of the commonest human viruses. The most interesting property of EBV is that both *in vitro* and *in vivo* it infects and immortalizes human B lymphocytes which then grow indefinitely. (The symptoms of glandular fever represent the cellular immune response to immortalized B cells.) It is the investigation of this property, and the gene regulation that governs latent immortalization and reactivation, that is the basis of EBV research today.

On the face of it, the infection of B cells clearly underlies Burkitt's lymphoma, which is a B-cell tumour, although the malignant transformed lymphoma cells are in many ways distinct from *in vitro* immortalized B cells. The tumour is clonal both for the cells and for the viral genome, so the virus was probably present in the original B cell that underwent malignant transformation. This seems compelling evidence for a causal malignant transformation between virus and tumour. The argument is strongest if few normal B cells are infected in those who develop the tumour. If, as is generally the case, the proportion is less than 1 per cent, the evidence is very strong indeed. It is not known, however, whether the percentage is so low in African children. Although EBV is almost certainly a contributory cause of Burkitt's lymphoma in the high-incidence areas of Africa it certainly is not a sufficient one (EBV-negative cases of Burkitt's occur all over the world). The effects of malaria on the immune system seem to be an essential factor — the true explanation of Burkitt's original observation of a climatic relation — and the tumour cells also possess a translocation which deregulates expression of the proto-oncogene *myc*. The translocation of *myc* is probably the transforming event. The role of immune surveillance is underlined by recent reports of true EBV-positive Burkitt's lymphoma in AIDS patients in the United States.

As a medical problem, Burkitt's lymphoma is hardly comparable with nasopharyngeal cancer, a disease which is most important in South-East Asia, with an incidence of 10–20 cases per 100,000

population a year. All tumour cells seem to contain virus, but the same reservations hold because nearly everyone has EBV in parts of their oropharynx. It seems that other factors must also be involved, for example, diet.

Following their original support for Epstein in the early 1960s, the CRC continued to support him in his pursuit of an anti-EBV vaccine as well as supporting much other EBV research. Vaccine research has concentrated on the glycoprotein antigen gp340 found on the viral envelope. The next step is to discover whether this molecule will evoke an immune response in man.

From a medical point of view, taking a

shot at producing a vaccine and going to field trials must be the priority for an organization whose *raison d'être* is cancer control. However, the significance of EBV goes far beyond this. It is a particularly valuable and interesting system in which to study virus latency and reactivation, exactly how the virus immortalizes the B cells, and it may shed light on more general aspects of cellular gene expression. Next week's conference should, with luck, bring us closer to understanding these questions. □

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## QUANTUM ELECTRONICS

# Interference rules the waves

Gerhard Fasol

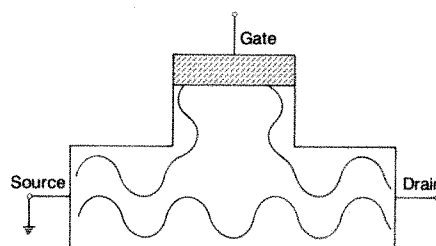
QUANTUM mechanics shows that electrons are waves. On the other hand, to explain conventional transistors, electrons are treated as classical particles propagating by diffusion. Thus there is usually no need to take the quantum-mechanical nature of electrons into account in modelling conventional devices. However, the wave character of electrons is unambiguously demonstrated by various experiments including tunnelling through potential barriers, which would be impenetrable for particles governed by classical physics, interference and diffraction. It is only recently that electron interference phenomena have become important in solid-state physics. Thus proposals for transistors based on quantum interference, as exemplified by an ingenious new design by F. Sols *et al.*<sup>1</sup>, are stimulating much interest.

In principle, transistors based on quantum interference are very attractive propositions, because of their very high expected speed. Thus they would be candidates for use in ultrafast computers, high-capacity telephone exchanges or satellite communication systems, for example. Unfortunately, present-day technology restricts such applications to extremely low temperatures (below 1 K). Thus conventional liquid helium cooling is not sufficient — a 'dilution' refrigerator is also needed. A common driving force of research on new types of transistors is to look for operating principles which use phenomena other than diffusion and charge-density modulation, which are both inherently slow. Deformations of wavefunctions and wave interference, on the other hand, are very much faster phenomena.

Maybe the most fundamental demonstration of the wave character of electrons is the generation of electron interference patterns, or electron holograms<sup>2</sup>. Electron

holography has recently been investigated very thoroughly by Akira Tonomura *et al.*<sup>3</sup> at the Hitachi Central Research Laboratory. Their experiment is based on the same principle as optical interference: an electron beam is split into two parts; the two partial waves then follow different paths and are subsequently allowed to interfere. The interference pattern is a consequence of the different phase of the two partial waves. In this way, magnetic flux patterns in superconductors or other structures which change the phase of an electron wave can be investigated.

Interference is well known for light waves: Newton's rings or the holograms used as a security device on credit cards are some well-known examples. A crucial condition for the observation interference phenomena is that the two beams interfering are coherent. This means that there must be a constant phase relationship between the two interfering partial waves over a time span corresponding to the difference in the path length of the two beams. In solids, it is not clear initially whether electron interference phenomena should ever be active, or indeed whether



The novel transistor proposed by Sols *et al.*<sup>1</sup> relies on interference between electron waves passing directly between drain and source and those diffracted via the stub. Applying a voltage to the gate changes the effective length of the stub, so that the waves can be made to interfere constructively (as shown) or destructively.

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experiments can be constructed where they should be observable. This is because in an ordinary semiconductor or metal, electrons collide continually with lattice vibrations, impurities, dislocations and other lattice imperfections. In addition, electrons collide with each other. Thus under normal conditions the coherence length is expected to be much too short to observe any interference phenomena.

But recently it has become clear that elastic scattering (impurity scattering, for example) changes the phase of an electron wave in a reproducible manner. Thus the observation of electron interference is limited only by inelastic electron-electron scattering and by phonon (lattice-vibration) scattering, which can be reduced by cooling the sample to extremely low temperature. The phase coherence length for electrons in GaAs heterojunctions is of the order of 5  $\mu\text{m}$  at 0.1 K, decreasing rapidly as temperature increases (ref. 4; also D. Wharam, personal communication). Thus, using current knowledge, any possible device based on quantum interference of electrons would have to be operated at extremely low temperatures and using dimensions at the limit of today's technology. Nevertheless, solid-state physics has been a source of exciting surprises recently: the discoveries of the quantum Hall effects (see my earlier News and Views article<sup>5</sup>), or high-temperature superconductivity, both rewarded by Nobel Prizes, were not at all expected before their experimental discovery. Therefore we should not have any prejudices in this presently very exciting field.

Transistors (core components occurring millions of times in any electronic equipment) are three terminal devices. In one particular class of transistors there is a current entering at one terminal and leaving at another. A third terminal controls this current: a large change in current through the first two terminals occurs if a small voltage (or a small current) at the third terminal is varied. In an intriguing new suggestion for a quantum interference transistor<sup>1</sup>, F. Sols *et al.* propose to use the wave character of electrons for transistor action. The authors investigate theoretically the propagation of electron waves through a T-junction-type waveguide, where a stub of variable effective size is attached to an electron channel. Model calculations show that the transmission coefficient through the channel depends strongly on the size of the stub (see figure). The effective size of the stub can be varied by changing the voltage applied to a gate on the stub. Thus such a device can be used as a transistor.

Tunnelling is another manifestation of the wave character of electrons: currents can pass through thin regions of material classically forbidden to them. M.A. Reed *et al.*<sup>6</sup> have just demonstrated the operation of a 'quantum resonant tunnelling transistor' in which the strength of the tunneling current between two terminals can be modulated strongly by a voltage applied to a third terminal. The essential physics of the effect was recently described in News and Views by Luryi<sup>7</sup>.

Other suggestions of unconventional transistor principles include that by Sakaki<sup>8</sup>, in which the wavefunction of electrons is switched between high and low mobility states — a velocity-modulation transistor. In this device, applying a voltage distorts the wavefunction of the electrons in a layered transistor structure perpendicular to the plane in which electrons are confined. Thus the electrons can be deformed towards the Coulomb scattering centres, reducing their mobility; or they can be pushed away from the scattering centres, increasing their mobility. Such a transistor works by controlling the velocity of electrons. This is expected to be inherently faster than the usual modulation of the charge density.

Another proposal relies on the diffraction of electron waves from surface gratings<sup>9</sup>. In this device electrons are confined to a two-dimensional channel and exposed to a periodic modulation of the thickness of the well or of a potential. For a modulation of the period  $a$ , electrons with wavelength  $\lambda = a/z$  (or close to it) will be reflected off the periodic grating. This effect is analogous to the Bragg reflection of light waves or X-rays. Thus the speed of electrons of a particular kinetic-energy range will be strongly affected by the grating. 'Negative differential resistance' can be achieved — a phenomenon whereby the current decreases with applied voltage in certain ranges. In a transistor structure, an applied voltage would change the depth of modulation and thus the velocity of the electron waves. □

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## PLANETARY ATMOSPHERES

# Impacts giveth and impacts taketh away

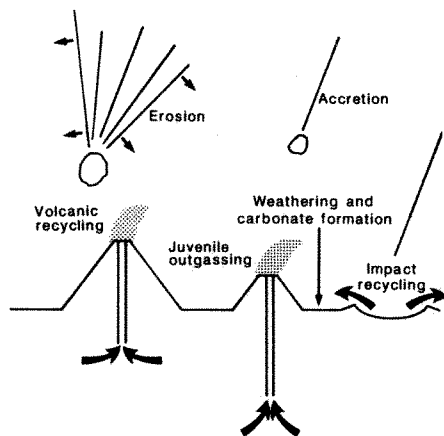
William B. McKinnon

COMETS have often been called upon to deliver life-giving volatiles and organic matter, early in the history of the Solar System, to the presumably barren surfaces of the terrestrial planets. Now, new research by Melosh and Vickery, on page 487 in this issue<sup>1</sup>, makes explicit the disquieting notion that bombardment by comets and asteroids can also erode, or even completely strip off, an atmosphere. The authors show by a simple model that Mars, with its relatively low gravity, is particularly susceptible. Working back in time, they find that Mars may have had an atmosphere with a surface pressure more like that of Earth at present. This idea has great scientific appeal as many lines of evidence point to a more clement Mars 3.8 thousand million or more years ago.

The idea that impacts can remove mass from an atmosphere is not new<sup>2</sup>, it is simply not well developed<sup>3,4</sup>. Melosh and Vickery have modelled the process numerically, but find that its essentials can be described by a simple analytical scheme. The specific mechanism they study is the interaction of the vapour cloud, produced when the impactor strikes the planetary surface, with the atmosphere. Other mechanisms have been studied, such as the shock waves produced by atmospheric passage and the entrainment of air by

high-speed solid ejecta<sup>3,4</sup>. In both cases, though, workers agree that the amount of atmosphere ejected is not more than a few times the amount intercepted by the projectile on the way in, or by the ejecta on the way out. The beauty of the vapour-cloud model is that much of the impactor's kinetic energy is transformed into vaporized impactor and target. The expanding cloud can then do work on the entire atmosphere in its line of sight.

Two criteria must be met for the expanding vapour cloud to blast the entire atmosphere in its sight to infinity. First, the impact must be fast enough for the vapour to expand at a speed greater than the planet's escape velocity, taking into account the considerable energy required for vaporization. Second, that the vapour mass must exceed the atmospheric mass in question. This is essentially a simple momentum balance. The characteristic sizes of rock impactors that can remove the entire air mass above a plane tangent to the surface are then roughly 3, 13 and 70 kilometres in diameter for Mars, Earth and Venus, respectively. Given these numbers, it is apparent that the atmospheres of all these worlds have been stable against impact erosion over most of geological time. That is, the number of impacts of this size are not great over a



Schematic diagram of the principal sources and sinks for  $\text{CO}_2$  in the early history of Mars.

few thousand million years at current cratering rates. The situation was entirely different during the first 500 million years or so of the Solar System, when the impact flux was orders of magnitude higher.

During the era of heavy bombardment, the authors show that the characteristic time for the martian atmosphere to be completely stripped is under 100 million years. Complete stripping is at least theoretically possible, because as the mass of the atmosphere declines, more numerous (and frequent) smaller impactors can erode it. Turning the problem around, Melosh and Vickery integrate backwards in time, undoing the effects of impact erosion. Using the agreed bombardment history for the inner Solar System (see the News and Views article by G. R. Stewart<sup>5</sup>), they show that Mars should have had a relatively thick atmosphere early in its history, barring other significant additions and subtractions. The atmosphere may even have been sufficient to allow running water on the planet's surface.

Impact erosion is clearly an important process in early planetary atmosphere evolution. The challenge ahead is to fit it into the general context. Atmospheres are, after all, not static entities. Earth's oxygen and nitrogen are maintained by living organisms. On Mars the situation is not as clear, but much research has focused on plausible histories for martian  $\text{CO}_2$ . The figure illustrates most of the important sources and sinks for  $\text{CO}_2$  on early Mars. Outgassing from the mantle is considered to be the ultimate source as the atmosphere is secondary, and is not derived directly from the solar nebula<sup>6</sup>. Comparison with the  $\text{CO}_2$  and carbonate budgets of the Earth and Venus suggests that Mars could easily derive an atmosphere with a pressure of many bars from this source.

Keeping it is another matter. Weathering of surface rocks and carbonate formation are estimated to cause the atmosphere to collapse on a timescale of  $10^7$

years according to Pollack *et al.*<sup>7</sup>. These authors invoke volcanic recycling to continually reflux  $\text{CO}_2$  back into the atmosphere. Mars was very active volcanically in early times, but as this activity waned, the atmosphere more-or-less irreversibly recombined with the surface. It may have halted only when the surface pressure reached the triple point of water (about 6 millibar), below which water will not stably exist, even transiently, and carbonate formation would cease<sup>8</sup>. Ground-based spectroscopic detection of the carbonate-bearing mineral scapolite in martian dust and soil has been reported recently<sup>9</sup>. (It is interesting to note that without life, Earth's atmosphere would also recombine with surface rocks, possibly leaving our planet in a Mars-like state, with only a thin, cold atmosphere of argon and  $\text{CO}_2$ .)

Impacts could have several effects on the early atmosphere (see figure). One is impact erosion. Impactors could also be a source of atmosphere if they are moving slowly enough. This is fine for asteroids, but Melosh states that comets generally move so fast that the vapour plume produced cannot be retained on Mars or the Earth (personal communication). Thus the idea that the Earth owes its oceans to cometary bombardment long ago may have to be abandoned. Impacts on Mars could also exhume and release carbonates. Schultz notes that erosion was much more intense on Mars before the formation of the last great impact basin, Argyre, than after<sup>10</sup>.

Perhaps the collapse of the martian atmosphere (recombination with the surface) was more tied to the impact history of Mars than to its volcanic history. Whatever the controlling factors, impact erosion is expected to occur, and may solve some old mysteries. Mars is depleted in argon compared to Earth and Venus, and this has been used to argue that Mars was less efficiently outgassed. Preferential impact erosion of the martian atmosphere may make this unnecessary<sup>1</sup>. Mars may be as efficiently outgassed as Earth, and thus may have released to its surface as much or more water per gram as Earth. □

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## Spinning in space

THE traditional space rocket ejects a stream of hot combustion gases. This is almost the only way of generating the huge but short-lived thrust needed to lift a spacecraft away from the Earth's surface. But once in space, a satellite or probe could be just as well manoeuvred by a very small thrust applied for a very long time. One possibility is to exploit solar or nuclear power to accelerate a continuous exhaust stream of electrons and heavy ions. But Daedalus has another suggestion. His new rocket motor doesn't eject any kind of gas or plasma. Instead it fires out a *solid* exhaust.

His ingenious propulsion system melts-spins a glass or polymeric material into a transparent fibre, writes alternate positive and negative charges on it from electron and ion sources, and then accelerates it to high velocity in an electrostatic linear motor. No net charge accumulates on the spacecraft, as the ejected fibre is on average electrically neutral. And in the microgravity and hard vacuum of space, it could be drawn to extremes of small diameter, high tenacity and high final velocity. Daedalus calculates that a 0.5-micrometre fibre ejected from a spacecraft at 4 km per second would generate about 10 millinewtons of continuous thrust from a few watts of power, easily drawn from solar panels. Over the months of a long space mission, this could build up a useful velocity increment, or make possible delicate manoeuvring, for a tolerable loss of mass. And as a final brilliant stroke, Daedalus will use the ejected fibre as an optical communication channel to link the spacecraft back to Earth.

The 'Space Spider' poses some intriguing problems. DREADCO's mathematicians are already wrestling with the gravitational dynamics of the ejected fibre, so as to establish trajectories for the Spider which will not stretch or break its ever-extending fibre. This would certainly break at the Earth end if any attempt were made to lead it down through the atmosphere. It will have to be anchored to a command satellite, orbiting in a plane normal to the Spider's trajectory: this would waggle the fibre but would not stretch or entangle it.

One serious snag is that few optical fibres are usefully transparent over more than 100 km or so. So Daedalus, cunningly, plans to equip his space fibre with distributed optical gain. DREADCO's chemists are devising super-radiant glasses which will lase in the sunlight of space. An optical pulse passing down such a glass fibre will be continuously amplified, and will travel unattenuated for any distance.

The Space Spider will open up whole new strategies of secure-communication deep-space probes. Sadly, only one or two can be flying at any one time, or they will surely get tangled up. David Jones



# Regulation of kinase activity

**SIR**—In a recent News and Views article, Hardie<sup>1</sup> described the emerging evidence that protein kinases can be inhibited by interacting with substrate-like sequences. These sequences are present either within the same molecule, as in kinase C, or in a different molecule, as in the regulatory subunits of cyclic AMP-dependent kinases. I would like to point out that an inhibitory role of amino-terminal sequences over the carboxy-terminal catalytic domain has also been shown for p60<sup>v-src</sup>, a member of the non-receptor family of tyrosine kinases<sup>2</sup>. The mechanism whereby this negative regulation occurs has not yet been addressed, although studies performed on p60<sup>c-src</sup>, the cellular (c) homologue of the viral (v) protein, support the idea of substrate-like sequences being involved.

Amino-acid positions 90 and 92 of p60<sup>c-src</sup> are tyrosine residues, one of which is surrounded by acidic amino acids as are many tyrosine-kinase target sequences (see figure). These sequences are located in the amino-terminal regulatory domain of the molecule within a stretch of about 50 amino acids that are conserved among all the members of the *src* family. This region has been called the SH3 or A box<sup>3</sup> and it is not present in the receptor class of tyrosine kinases.

Activated p60<sup>c-src</sup> molecules, such as those bound to middle-T antigen of polyomavirus, or those present in certain neuroblastoma cell lines, are phosphorylated on tyrosine at their amino termini<sup>4,5</sup>. Based on their neighbouring sequences, tyrosine residues 90 and 92 represent the most likely targets of phosphorylation within this amino-terminal region. An

interpretation of these results is that in the off state the catalytic carboxy-terminal domain interacts with the amino-terminal substrate-like sequences. Phosphorylation of the amino-terminal site would only occur when the kinase is activated, presumably by an induced conformational change. Phosphorylation at these sites may allow maintenance of the on state.

A similar effect is observed with certain amino-acid substitutions of p60<sup>c-src</sup>. These are Gly 63→Asp, Arg 95→Trp, and Thr 96→Ile, which are present in the viral protein p60<sup>v-src</sup>, and are sufficient to increase the specific activity of the kinase<sup>6</sup>. The recombinant chimaeras between v-*src* and c-*src* used in these studies<sup>7</sup> are shown schematically in the figure. Among these three mutations, the crucial one is likely to be Arg 95→Trp, because Gly 63→Asp when present alone in p60<sup>c-src</sup> is silent, and Thr 96→Ile is not conserved in other strains of Rous sarcoma virus. Arg 95 is three residues away from the tyrosine-phosphorylation site and its presence could weaken the interaction with the kinase domain, thereby releasing the inhibition and allowing constitutive activation of the kinase.

A more rigorous proof that Arg 95 lies within a kinase regulatory domain has been obtained by Potts *et al.*<sup>7</sup>, who find that the Arg 95→Trp mutation alone is sufficient to activate p60<sup>c-src</sup>. In addition, this group and also L. Fox, K. Frost and J.S. Brugge (personal communication), have shown that mutations at either tyrosines 90 or 92, or the deletion of amino acids 92–95, activate the transforming potential and the kinase activity of p60<sup>c-src</sup>. Thus, it is possible that an intramolecular

negative control of kinase activity among the *src* family resides within substrate-like sequences outside the catalytic domain.

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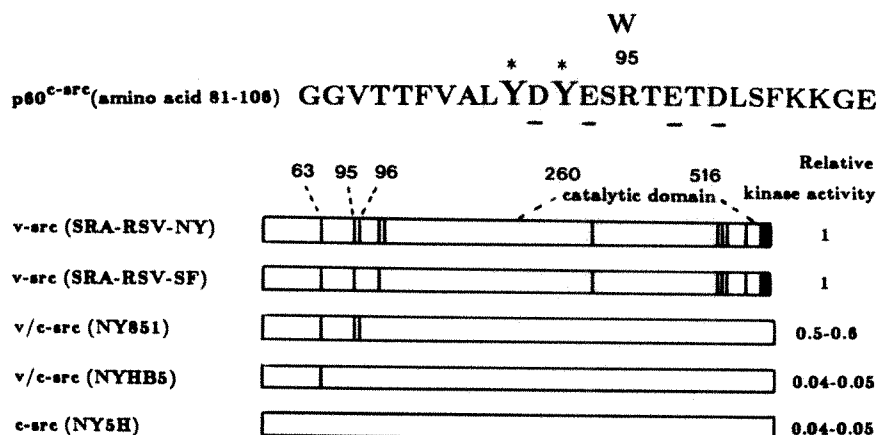
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## Tale of two serines

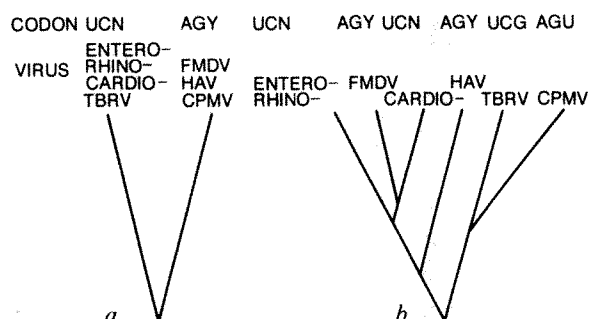
**SIR**—In a recent paper<sup>1</sup>, Brenner used the fact that serine is encoded by two non-linked codon types, UCN and AGY, in conjunction with his observation that within several enzyme families catalytic serine residues have different codon representations, to propose that these serines evolved convergently by single substitutions in cysteine or threonine codons (UGY and ACN, respectively), the latter being catalytic residues in ancestral enzymes of each class. This proposal, however, fails to account for the serine codon representations of two groups of proteins.

First, the chymotrypsin-like proteases from *Streptomyces griseus*, SGPA and SGPB (ref. 2): in these enzymes, the catalytic serine residues are encoded by AGU and UCC, respectively, but the 61 per cent identity of amino acids between SGPA and SGPB, and their similar sizes, make it highly improbable that they belong to two different phylogenetic lineages, as predicted by Brenner's hypothesis. Second, the viral replicative proteins containing the widespread nucleoside-5'-triphosphate (NTP)-binding motif (refs 3–5) Gly-X-X-X-Gly-Lys-Ser/Thr (GXXXXGK S/T) where X is any amino acid: as shown in the figure and table, the lineages of the GKS-containing proteins of picornavirus, comovirus and nepovirus derived from serine codon types are incompatible with the phylogeny arising from sequence comparisons (compare *a* and *b* in the figure). Moreover, the conspicuous absence in this family of a GKT-containing protein is at odds with Brenner's additional suggestion that, in the case of the NTP-binding motif, threonine might be an evolutionary intermediate between the two kinds of serine, rather than their predecessor. It seems to be the case, in the proteins of this family, that threonine is not acceptable in the NTP-binding motif and that evolutionary transitions between UCN and AGY



Amino acids 81 to 106 of p60<sup>c-src</sup>. W, substitution of Arg→Trp as in p60<sup>v-src</sup>; asterisks are above tyrosines 90 and 92, underlines, acidic amino acids surrounding the tyrosine-phosphorylation sites. Lower panel, schematic diagram of various *src* proteins from: Schmidt Ruppel subgroup A Rous sarcoma virus, New York strain (SRA-RSV-NY); San Francisco strain (SRA-RSV-SF); chimaeric viral and cellular *src* recombinant viruses (NY851 and NYHB5); a non-mutated cellular *src* recombinant virus (NY5H). Mutations 63, 95 and 96 are indicated; vertical lines show amino-acid substitutions in other regions of the viral protein as compared with the cellular *src* protein. The relative kinase activity was measured by *in vitro* phosphorylation of enolase as previously described<sup>5</sup>.

Phylogeny of putative NTPases of picornavirus, comovirus and nepovirus. *a*, Phylogenetic scheme derived from the data of the table according to Brenner's hypothesis; *b*, phylogenetic tree generated by comparison of amino-acid sequences of evolutionary conserved segments of putative NTPases using a rate-independent distance matrix method<sup>7,8</sup>. Only the branching order is shown; the branch lengths were chosen arbitrarily. The branching order of this tree is identical to that generated for viral RNA polymerases<sup>9</sup> and for capsid proteins<sup>10</sup>, and presumably reflects the phylogeny of viral genomes as a whole. Codon representations of serine in the GKS consensus are shown. Where, in a group of viruses, only one codon series is utilized, the branching order was not further specified.



codons occur without GKT intermediates.

In two other families of viral GKS/T-containing proteins serine is encoded almost exclusively by UCN, with AGY occurring only once (see table). Again, it is unreasonable to suppose that the potexvirus gene containing AGU originated from a separate line of descent. Both these families, however, include GKT-containing proteins seemingly making the evolution of differentially encoded serines through threonine intermediates a possi-

bility. Nevertheless, it seems that if this occurred then it did so only rarely.

Although our survey of serine codon usage in putative viral NTPases does not support Brenner's hypothesis, it exposes some intriguing variations in the evolutionary mechanisms of different phylogenetic lineages. At least two mechanisms may be invoked to explain how the UCN to AGY transition occurs without loss of serine at the enzyme active site. The first of these requires the simultaneous change of two adjacent bases. Given the high error rate of RNA replication<sup>6</sup>, this mechanism is more feasible for RNA viral genomes than for DNA genomes. An alternative mechanism involves the generation of a new serine codon next to the functionally important one (yielding a

GKSS sequence with the two serines encoded by codons of different series) followed by deletion of the original codon. This mechanism is equally feasible with DNA and RNA genomes and might operate wherever there is no strict constraint on the residue(s) next to the functional serine. Generally, understanding the evolutionary history of serine codons in catalytic centres of each enzyme class requires knowledge of its phylogeny derived from independent data.

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Serine codon representations in the nucleotide-binding motif of positive strand RNA viruses

Family of viral 'NTPases'	Consensus sequence	Serine (threonine) codons
<b>Family I</b>		
Alphaviruses	GKS	UCN
Coronaviruses	GKS	UCC
Furoviruses	GKS	UCN
Hordeiviruses	GKS	UCA
Tobraviruses	GKS	UCG
Potexviruses		
WC1MV p147	GKS	UCU
p26	GKS	UCU
PVX p165	GKS	AGU
p26	GKS	UCC
Tricornaviruses	GKT	ACN
Tobamovirus	GKT	ACC
Tymovirus	GKT	ACA
<b>Family II</b>		
Enteroviruses	GKS	UCN
Rhinoviruses	GKS	UCN
Cardioviruses	GKS	UCN
Aphtoviruses (FMDV)	GKS	AGY
Hepatitis A virus (HAV)	GKS	AGY
Comovirus (CPMV)	GKS	AGU
Nepovirus (TBRV)	GKS	UCG
<b>Family III</b>		
Potyviruses	GKS	UCN
Flaviviruses	GKT	ACN
Pestiviruses	GKT	ACA

For sources of sequence data see refs 4, 11 and 12. N, any nucleotide; Y, pyrimidine. The grouping of viral proteins containing the NTP-binding motif is according to refs. 4 and 12. Potexviruses (as well as furoviruses and probably hordeiviruses) have two putative NTPases each<sup>4</sup>. Different species of enterovirus and rhinovirus, and different strains of foot and mouth disease virus (FMDV) and HAV have either C or U in the third position; hence, N or Y is indicated, respectively.

## Telomere formation in yeast

**SIR**—We recently demonstrated that during formation of new telomeres in the yeast *Saccharomyces cerevisiae*, telomeric sequences are often transferred between DNA termini<sup>1</sup>. We argued that the most reasonable explanation for this transfer is recombination between DNA termini. In a recent News and Views article<sup>2</sup>, however, Szostak suggested that the telomere resolution reaction<sup>3</sup> (the cleavage between two blocks of telomeric sequences that are oriented as a head-to-head inverted repeat<sup>3,4</sup>) can provide an alternative explanation for our data, a possibility that can be addressed definitively by DNA sequencing. It is not clear to us why this possibility was raised because we stated<sup>1</sup> that our unpublished sequence data support the interpretation presented in the article; that is, the orientation of the transferred repeats is the same as that of the test termini (S.-S. Wang and V.A.Z., in preparation).

Although the sequence data eliminated the resolution model as an explanation for the telomeric transfer, we did not discuss these data specifically in terms of this model<sup>1</sup>. The resolution reaction never provided a likely explanation for our results because it requires three events: (1) circularization of linear plasmids bear-

ing telomeric repeats at each end; (2) asymmetrical resolution of the circles thus formed; and (3) telomere formation on an end with the telomeric repeats in the 'wrong'<sup>1,4</sup> orientation. Not only have none of these processes been demonstrated in yeast, but even symmetrical resolution is inefficient (~1 per cent per cell division)<sup>4</sup> compared with the sequence transfer we observe.

Because the resolution reaction is excluded unequivocally by DNA sequence data, telomere-telomere recombination remains the only reasonable explanation for the transfer of telomeric sequences that we have observed. Whether or not yeast exploits telomere-telomere recombination in the replication or maintenance of telomeres remains to be determined.

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## Saxtons River, Vermont


 **RENAL HEMODYNAMICS: INTEGRATIVE AND CELLULAR CONTROL MECHANISMS** June 11-16 Chairs: L. Gabriel Navar, Tulane University Medical School; Donald L. Marsh, University of Southern California. **Structure and Development.** W. Kriz, A. Evan, D. Abrahamson, D. Casellas, L. Barajas; **Cell Biology of the Renal Microvasculature.** D. Schlondorff, J. Kreisberg, M. Dunn, K. Kurokawa, J. Bonventre, A. Hassid, W. Schrier; **Responses of Vasculature to Extrinsic Perturbations.** W. Arendshorst, H. Kirchheim, G. Navar, A. Premen; **Assessment of Renal Microvascular Responses.** M. Steinhausen, R. Edwards, P. Carmines, D. Harder, J. Briggs; **Tubuloglomerular Feedback Mechanism Control.** J. Schnermann, D. Bell, E. Persson, B-E. Persson, J. Davis; **Control of Renal Vasculature by Angiotensin.** R. Blantz, L. Rosivall, J. Hall, B. Ballermann, B. Zimmerman; **Neural Control of Renal Vasculature.** G. DiBona, E. Johns, V. Kon, M. Wolgast, N. Moss; **Regional Control of Intrarenal Hemodynamics.** F. Knox, R. Roman, S-Y. Chou, M. Sjoquist, R. Jamison; **Mathematical Modelling.** D. Marsh, L. Moore, K. Aukland, N. Holstein-Rathlou.


 **CELLULAR AND MOLECULAR GENETICS** June 18-23 Chairs: Gretchen J. Darlington, Baylor College of Medicine; Inder Verma, The Salk Institute. **Transcription Factors.** M. Rosenfeld, K. Calame, R. Tjian; **Organization and Action of Receptor Proteins.** K. Yamamoto, M. Johnston, C. Wu, J. Thorner; **Gene Regulation by Cytokines.** G. Darlington, G. Wong, J. Massague, G. Stark; **Tissue Specific Gene Regulation.** H. Blau, G. Schutz, R. Roeder, P. Gruss; **Human Disease and Gene Therapy.** S. Woo, L-C. Tsui, R. Mulligan, M. Capecchi; **Cell Cycle Regulation.** D. Nathans, D. Beach, E. Harlowe, A. Murray; **Post-Transcriptional Gene Regulation.** J. Ross, A. Jacobson, R. Klausner, E. Ehrenfeld; **Gene Regulation in Differentiation and Development.** C. Emerson, M. Karin, M. Kuehl; **Molecular Analysis of Oncogenes and Tumor Suppressors.** I. Verma, O. Witte, W-H. Lee, A. Berns.


 **BIOLOGY AND CHEMISTRY OF VISION** June 25-30 Denis Baylor, Stanford Medical School; Bernard Fung, University of California/Los Angeles. **Visual Pigments.** J. Nathans, G. Khorana, D. Oprian, C. Zucker; **Cyclic GMP Cascade of Vision - Chemistry.** L. Stryer, M. Chabre, T. Wensel, B. Fung; **- Physiology.** K-W. Yau, P. Detwiler, K. Nakatani; **Photoreceptors.** D. Bok, R. Molday, J. Besharse; **Ion Transport Proteins.** M. Applebury, D. Nicoll, R. Hurwitz; **Hereditary Retinal Degenerations.** D. Farber, W. Pak; **Visual Transduction - In Cones.** J. Hurley, C. Lerea, J. Beavo, L. Haynes; **- In Invertebrates.** J. Lisman, J. Brown; **Molecular Mechanisms of Synaptic Transmission.** J. Dowling, F. Werblin, E. Schwartz, A. Knapp; **Keynote Address: Retinitis Pigmentosa.** E. Berson.

 **UBIQUITIN AND INTRACELLULAR PROTEIN DEGRADATION** July 2-7 Chairs: Milton J. Schlesinger, Washington University School of Medicine; Alfred L. Goldberg, Harvard Medical School. **Genes and Their Expression.** A. Varshavsky, S. Jentsch, R. Vierstra, E. Knight; **Ubiquitin Enzymes I & II.** A. Hershko, K. Wilkinson, I. Rose, C. Pickart, A. Ciechanover, C-C. Liu, A. Haas; **Other Roles for Ubiquitin.** M. Schlesinger, D. Finley, J. Mayer; **Prokaryotic Proteolytic Degradation.** S. Gottesman, C. Gross, J. Little, C. Chung; **The Proteasome.** A. Goldberg; **Protein Breakdown - Regulation of Enzyme Levels.** M. Rechsteiner, P. Coffino, R. Eisenman, J. Richter; **- Regulation by Physiological Factors.** J. Dice, O. Scornik, M. Goodman, J. Ellinger; **Proteolytic Degradation in Organelles.** R. Klausner, P. Stahl, T. Braciale.

 **CALCIUM AND CELL FUNCTION** July 9-14 Chairs: Anthony R. Means, Baylor College of Medicine; Kevin Campbell, University of Iowa. **Structure/Function of Ca<sup>2+</sup> Binding Proteins.** R. Kretsinger, K. Beckingham, J. Putkey, D. MacLennan; **Gene Regulatory Mechanisms.** M. Rosenfeld, H. Kronenberg; **Regulation in Excitable Cells.** R. Tsien, L. Birnbaumer, S. Snyder, P. Conn; **Sequestration and Release Mechanisms.** P. Volpe, E. Carafoli, T. Vanaman, L. Jones; **The Protein Phosphorylation Cycle.** A. Nairn, H. Hidaka, H. Schulman, C. Klee; **Regulation of Responsiveness and Contractility.** H. Rasmussen, R. Murphy, J. Bryan; **Emerging Ca<sup>2+</sup> Control Systems.** M. Crumpton, D. Storm, J. Glenney, K. Suzuki; **Growth and Development.** R. Steinhardt, R. Baserga, W. Klein, M. Inouye; **Genetic Analyses of Ca<sup>2+</sup> Binding Proteins.** C. Rasmussen, T. Davis, C. Kung.

 **CELLULAR AND MOLECULAR STUDIES IN BONE MARROW TRANSPLANTATION** July 16-21 Chairs: Brian Richard Smith, Yale University School of Medicine; Steven J. Burakoff, Dana-Faber Cancer Institute. **The Major Histocompatibility Complex.** J. Hansen, J. Strominger, R. Flavell; **T and NK Cell Ontogeny and Function.** S. Burakoff, J. Ledbetter, R. Miller, S. Strober; **Graft Rejection and Graft Tolerance.** R. O'Reilly, M. Bennett, D. Sachs; **Graft Versus Host Disease I & II.** R. Parkman, A. Abbas, D. Vallera, B. Smith, J. Ferrara, R. Korngold, A. Steinberg; **Regulation of Hematopoiesis.** D. Nathan, I. Bernstein, D. Linch; **Gene Transfer Therapy.** J. Rapoport, A. Neinhuis, J. Barranger, E. Gilboa; **B Cell Ontogeny and Function.** B. Smith, P. Lipsky, K. Denis, D. Well; **Accessory and Endothelial Cell Function.** J. Pober, R. Geha.

 **LYMPHOCYTES AND ANTIBODIES** July 23-28 Chairs: Carol Cowing, Medical Biology Institute; David Parker, University of Massachusetts. **MHC and T Cell Recognition.** L. Glimcher, L. Matis, G. Fathman; **Mechanisms of Self-Tolerance.** H. von Boehmer, C. Goodnow, D. Lo; **T Cell Phenotypes.** K. Bottomly, S. Shaw; **Antigen Processing Pathways.** M. Bevan, P. Allen, K. Fischer-Lindahl; **Early B Cell Development.** N. Rosenberg, P. Kincade, R. Wall; **Regulation of Isotype Switch.** R. Koffman, P. Rothman, J. Stavnezer; **Ontogeny and Function of (g)/(d) Cells.** H. Wotis, J. Bluestone, M. Brenner; **Lymphocyte Activation.** G. Crabtree, W. Leonard, G. Nabel; **Immunopathogenesis of HIV.** L. Chess.

 **REGULATION OF ENERGY BALANCE AND NUTRIENT PARTITIONING** July 30-August 4 Chairs: M. R. C. Greenwood, Vassar College; Ahmed Kissebah, Medical College of Wisconsin; Samuel W. Cushman, NIDDK/NIH. **Modulating Sensory and Metabolic Factors.** A. Sclafani, J. Stern, J. Vasselli, N. Rowland, A. Drewnowski, B. Rolls; **Three Integrative Perspectives on Energy Balance.** E. Horton, J. Hirsch, E. Jequier, G. Bray; **Caloric Balance.** E. Ravussin, J. Hill, D. Schoeller, A. Prentice, S. Roberts, B. Horowitz, J. Wilmore; **Nutrient Partitioning and Utilization - Within Organs.** R. Leibel, R. Martin, J. Kinney, B. Levin, R. Eckel, J. Kinsella; **-Among Different Adipose Tissues.** S. Cushman, S. Fried, U. Smith, A. Kissebah, M. Rebuffe-Scrive, M. Lavau, L. West; **Systemic and Cellular Integration.** G. Wade, T. Bartness, A. Campfield, S. Cushman, C. Landos; **Molecular and Genetic Aspects.** M. Greenwood, M. Scholtz, P. Belfrage, B. Spiegelman, G. Ringold, D. Ricquier; **Keynote Address: Substrate and Hormonal Regulation of Apo-Lipoprotein mRNA and Lipid Partitioning.** H. Brewer, Jr., B. Hansen; **Clinical and Medical Aspects of Metabolic Dysregulation.** S. Heymsfeld, R. Atkinson, G. Reaven, P. Bjorntorp, J. Brunzell, J. Gibbs.

# RESEARCH CONFERENCES

**THE NEUROBIOLOGY OF CNS INJURY** August 6-11 Chairs: Alan I. Faden, University of California/San Francisco; Wise Young, New York University Medical Center. **Methodological Issues.** C. Hsu, J. Lightall, W. Pulsinelli, D. Choi, R. Traystman, T. Colton; **Behavioral Evaluation.** J. Wrathall, M. Goldberger; **Blood Flow/Metabolism.** W. Obrist, M. Ginsberg, W. Powers, K. Welsh; **Neurochemistry Methods/Approaches.** S. Panter, J. Prichard, B. Siesjo; **Neurochemical Factors in Secondary Injury.** B. Meldrum, P. Demediuk, M. Braugher, T. McIntosh, R. Kraig, W. Young, R. Vink; **Pharmacology.** A. Faden, E. Hall, J. Holaday, R. Miller, J. Zivin, B. Lyeth, R. Simon; **Histological Methods.** J. Povlishock, L. Noble; **Physiological Methods.** C. Tator, A. Blight.

**MOLECULAR NEUROGENETICS** August 13-18 Chairs: J. Gregor Sutcliffe, Research Institute of Scripps Clinic; Allan Tobin, University of California/Los Angeles. **Cell Specification - Vertebrates.** M. Kennedy, G. Travis, S. Heinemann; **- Invertebrates.** L. Zipursky, S. Crews, S. Benzer; **Genetic Manipulation.** G. Evans, C. Cepko, M. Capecchi, T. Claudio; **Genetic Disorders.** E. Ginns, P. Ray, E. Gershon, U. Franke; **Cell Specializations: Vertebrates.** A. Tobin, R. Milner, D. Anderson, R. McKay; **Behavior Genes: Invertebrates.** J. Hall, J. Dunlap, J. Fisher, C. Zuker; **Cell and Molecular Biology of Learning.** P. Hyslop, S. Rose, D. Clayton; **Neural Development -Invertebrates.** M. Chalfie, R. Horvitz, M. Young, J. Carlson; **- Vertebrates.** R. Mullen, K. Herrup, M. Brennan, R. Brackenbury, M. Chai.

## Copper Mountain, Colorado

**BIOCHEMICAL AND BIOPHYSICAL MECHANISMS IN GRAVITY RESPONSES** June 25-30 Chairs: Carl Leopold, Cornell University; Marc Tischler, University of Arizona. **Evolution of Gravity Systems.** D. Wolgemuth, F. Sack; **Sensing Mechanisms.** R. Hertel, R. Wayne; **Ion Pumps and Electric Regulation.** T. Bjorkman, W. Schreurs, R. Nuccitelli, K. Rathor; **Environmental Sensing Systems.** M. Jaffe, K. Lohman; **Ionic Regulation.** S. Roux, R. Cleland, D. Perdue; **Hormonal Involvements.** M. Evans, S. Max, E. Holton, C. Leopold; **Growth and Development.** E. Holton, M. Tischler, R. Levine; **Genetic Regulation.** K. Poff, M. Marron. **Overview.**

**GASTROINTESTINAL TRACT III: REGULATION OF ORGAN/CELLULAR FUNCTIONS** July 2-7 Chairs: Jackie D. Wood, Ohio State University; Gilbert A. Castro, University of Texas HSC/Houston. **Cell/Molecular Mechanisms of Development.** D. Alper, B. Ponder, J. Gordon, M. Neutra; **Cell-to-Cell Interactions in Regulation of Epithelial Development.** J. Jameson, P. Ekblom, M. Bernfield, N. Gilula, J. Madara; **Oncogene Expression and Regulation of Tumor Development.** D. Poldosky, S. Hamilton, R. Bernards, R. Coffey; **Epithelial Transporters, Channels and Pumps.** L. Reuss, N. Wills, Y. Segal, S. Schultz, M. Cerreijido; **Dietary and Hormonal Regulation.** S. Henning, O. Koldovsky, R. Grand, T. Goda, N. Davidson, C. Haffen; **Neural Regulation.** H. Cooke, A. Surprenant, M. Gershon, O. Lundgren; **Mechanisms of Regulation of Gastrointestinal Musculature.** J. Szurszewski, J. Singer, G. Makhlof, K. Sanders; **Eicosanoid Messengers.** M. Wasserman, A. Robert, S. Konturek, D. Rachmilewitz, P. Smith; **Immunological Trigger Systems.** G. Castro, J. Bienenstock, M. Perdue, G. Gall, D. Powell.

**GENETIC RECOMBINATION AND GENOME REARRANGEMENTS** July 9-14 Chairs: John Wilson, Baylor College of Medicine; Richard Kolodner, Dana-Faber Cancer Institute. **Genome Rearrangements.** R. Rothstein, K. Blackwell, E. Selker; **Physical Structures in Recombination.** P. Modrich, D. Lilley, J. Griffith; **Genetic Control.** R. Esposito, G. Smith, J. Haber; **Genome Remodeling in Mammals.** C. Caskey, D. Miller; **Catalysis of Homologous Pairing.** M. Cox, C. Radding, D. Camerini-Otero; **Site-Specific Recombination.** A. Landy, N. Grindley, R. Hoess, H. Nash; **Nucleases Involved in Recombination.** A. Clark, F. Heffron, S. Kushner; **Transposition.** N. Cozarella, K. Mizuuchi, N. Craig; **Mismatch Repair.** M. Lieb; **History of the DNA Heteroduplex.** R. Holliday.

**PROTEIN KINASES** July 16-21 Chairs: Perry J. Blackshear, Duke University Medical Center; Jackie D. Corbin, Vanderbilt University School of Medicine. **Opening Address.** Y. Nishizuka; **Mitogen and Cell-Cycle Dependent Protein Kinases.** J. Maller, M. Czech, R. Erikson, P. Nurse; **Protein Kinase C.** K-P. Huang, P. Blackshear, G. Nelsestuen, P. Parker, R. Davis, C. Harley; **Ca<sup>2+</sup>/Calmodulin Dependent Protein Kinases.** A. Nairn, J. Stull, M. King, A. Means; **Cyclic Nucleotide-Dependent Protein Kinases.** J. Corbin, B. Kemp, M. Zoller, J. Gold, J. Avruch; **Tyrosine Kinases: Viral/Cellular.** T. Hunter, O. Witte, T. Pawson, B. Sefton; **Receptors.** J. Avruch, R. Roth, G. Gill, L. Williams; **Protein Phosphatases.** P. Cohen, E. Fischer, T. Ingebritsen, R. Kincaid; **Plenary Session.** E. Krebs; **Protein Kinases and Gene Transcription.** D. Granner, J. Habener, M. Montminy.

**MICRONUTRIENTS: TRACE ELEMENTS** July 23-28 Chairs: Robert J. Cousins, University of Florida; Ananda S. Prasad, Wayne State University; Robert B. Rucker, University of California-Davis. **Absorption and Transport.** K. Smith, J. Glass, B. Lonnerdal, J. Turnlund, G. Brewer; **Workshop.** L. Schiff, D. Foster; **Gene Expression I.** J. B. Neilands, J. Gitlin, H. Nick; **Cellular Metabolism.** E. Harris, H. Cohen, R. Cousins; **Gene Expression II.** F. Sunderman, Jr., D. Hamer, R. Sunde, N. Amy; **Experimental Immunology.** P. Fraker, R. Winchurch, J. Prohaska, M. Failla; **Free Radicals.** S. Aust, J. Gutteridge, T. Bray; **Biological Effects.** R. Rucker, D. Baly, M. Korc, H. Lukaski; **Clinical Effects.** K. M. Hambidge, C. McClain, F. Nielsen, A. Prasad.

**NUTRIENTS AND GENE EXPRESSION IN CARCINOGENESIS** July 30 - August 4 Chairs: Willard J. Visek, University of Illinois College of Medicine; Lionel A. Poirier, National Center for Toxicological Pathology. **Gene Expression and Cancer: Perspectives.** J. Rowley, T. Waldman, T. Osborne, B. Ames; **Calories, Fat, and Gene Expression.** R. Hart, R. Eastbrook, D. Busbee, K. Randerath; **Oncogenes and Growth Control and Carcinogenesis.** D. Blair, M. Barbacid, M. Greenberg, H. J. Kung; **Methyl Deficiency and Biological Systems.** P. Newberne, L. Poirier, R. Hoffman, F. Feo; **Methylation of DNA.** J. Christman, R. Challet, S. Baylin, R. Perry; **Calcium, Cell Proliferation, Differentiation, and Carcinogenesis.** M. Lipkin, R. Wasserman, G. Stoner, H. Newmark, M. Wargovich; **Nutrients and Signal Transduction.** P. Blumberg, S. Joseph, R. Reed, M. Karin; **Hormones, Hormone Receptors, and Gene Expression.** J. Gustafsson, G. Norstedt, M. Rechler; **Vitamins' Trace Elements and Gene Expression.** L. DeLuca, P. Davies, B. Komm, M. Linder.

**PLANT GENE EXPRESSION** August 6-11 Chairs: Peter H. Quail, Plant Gene Expression Center; Michael Bevan, PBI Cambridge. **Mutant Isolation and Analysis I & II.** V. Walbot, H. Goodman, B. Baker, S. Dellaporta, J. Jones, C. Somerville, G. Fink, M. Freeling, M. Yanofski; **Floral Development.** M. Crouch, E. Coen, A. Clarke, S. Levings, S. McCormick; **Workshop: Transformation Methods.** R. Horsch, M. Fromm, K. Barton, K. Hinata, L. Herrera-Estrella, E. Picard; **Developmentally-Regulated Genes.** R. Goldberg, N. Raikhel, R. Beachy, D. Grierson; **Regulatory Molecules I.** D. Baulcombe, S. Theologis, R. Fischer, D. McCarty, M. Estelle; **Workshop: Novel Methods.** R. Horsch, I. Potrykus, J. Paszkowski, B. Hiatt, J. Haselhoff, S. Rogers; **Regulatory Molecules II.** H. Klee, T. Guilfoyle, J. Schell, W. Bruce; **Environmentally-Regulated Genes.** E. Tobin, A. Cashmore, P. Gilmartin, G. Coruzzi, W. Gurley; **Plant-Pathogen Interactions.** C. Lamb, C. Ryan, L. Willmitzer, J. Bennetzen, B. Staskawicz; **Keynote Address: LEA Proteins and their Genes.** L. Dure; **Agricultural Applications.** R. Fraley, J. Leemans, J. Bedbrook, B. Mazur.

**MOLECULAR MECHANISMS OF CARCINOGENESIS** August 13-18 Chairs: Michael J. Weber, University of Virginia School of Medicine; Michael W. Lieberman, Baylor College of Medicine. **Molecular Epidemiology.** W. Cavenee, R. White, E. Solomon or W. Bodmer, J. Minna, E. Fearon; **Viral Oncogenesis.** P. Howley, D. Lowy, A. Levine, F. Chisari; **Oncogene/Carcinogen Interactions in Experimental Neoplasia.** G. Bowden, M. Anderson, S. Garte, S. Sukumar; **Cellular Responses to Carcinogens and Genotoxic Agents.** J. Whitlock, J. Essigman, B. Ames, M. Gottesman; **Oncogene Structure and Function.** J. Parsons, C. Sherr, J. Brugge; **Signal Transduction.** M. Weber, R. Erikson, J. Gibbs, N. Colburn; **Gene Expression I & II.** M. Lieberman, H. Herschman, B. Crombrughe, R. Eisenman, B. Spiegelman, T. Curran; **Anti-Oncogenes and Tumor**

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B. Lachmann, University of Rotterdam (Ed.)

## **Surfactant Replacement Therapy**

in Neonatal and Adult Respiratory Distress Syndrome

1988. 130 figures. XXII, 372 pages. Hard cover DM 136,-. ISBN 3-540-18734-0

This book presents state-of-the-art information from the groups who have developed and used different types of surfactant. The latest advances concerning in vivo and in vitro evaluation of different types of surfactant as well as clinical consequences, such as changes in ventilator settings following surfactant replacement therapy and immunological questions, are discussed.

T. M. Schroeder-Kurth, University of Heidelberg; A. D. Auerbach, The Rockefeller University; G. Obe, University of Essen (Eds.)

## **Fanconi Anemia, Clinical, Cytogenetic and Experimental Aspects**

1989. 70 figures. Approx. 290 pages. Hard cover DM 138,-. ISBN 3-540-50401-X

This monograph represents the first attempt to gather all aspects of Fanconi's anemia in one source. The difficulties in differential diagnosis and treatment are covered; and the outlook for cure via bone marrow transplantation is included. This overview will interest specialists in human genetics as well as those dealing with this disease.

G. Valenti, Parma (Ed.)

## **Psychoneuroendocrinology of Aging**

**Basic and Clinical Aspects**

1989. Approx. 175 pages. (FIDIA Research Series, Volume 16). Hard cover DM 74,-. ISBN 3-540-96943-8

This book summarizes reports delivered at a recent workshop at the University of Parma. In the first part, the psychoneuroendocrine pattern in the so-called normally aging brain is described. In the second part, the psychoneuroendocrine pattern is depicted for the most stressful expressions of pathological aging such as the Alzheimer type, multi-infarct dementia, Parkinson's disease, depression and memory impairments.

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# Trials of a lifetime

Alex Comfort

**The Retardation of Aging and Disease by Dietary Restriction.** By Richard Weindruch and Roy L. Walford. *Charles C. Thomas, Springfield, Illinois: 1989. Pp. 436. \$69.75.*

ONE of the few things we know — and have known for half a century — about the ageing process in mammals is that it can be slowed by caloric restriction. McCay's original finding has been repeatedly challenged and repeatedly re-verified, not only in mammals, the organisms which really preoccupy experimental gerontologists, but also in fish, invertebrates and test organisms generally. The apparent slowing, moreover, is not just a reduction in the diseases of overnutrition. Rather it is a moving to the right of the curve of survival, and the curve seems to move all identifiable landmarks with it: one would confidently predict that pedestrians subject to dietary restriction (DR) would have a peak for road fatalities at a higher age than those who are non-restricted.

Weindruch and Walford have produced a comprehensive book on this entire question. It powerfully concentrates the mind, notably by making it impossible to avoid asking why more effort has not been concentrated on assessing this effect in human beings. The mode of action of DR remains obscure — there are cellular, programme delay, error accumulation, neuroendocrine and immunological hypotheses, and the literature and evidence for all of them is painstakingly reviewed here for the first time. What precisely happens to molecular, physiological and immunological parameters is reviewed in equal detail, a fairly massive undertaking in view of the large range of organisms and the desultory character of much of the research. The purpose of this overview is to prepare us to ask ourselves whether, on the evidence, DR should work in human beings, and, if so, why no concerted attempt is being made to test it.

One can think of a number of answers to the last part of the question — actuarial studies on diet are by nature a two-lifetime project unlikely to attract funding sources wedded to instant gratification; and non-actuarial biomarkers, as the Hiroshima project investigators found, are quite difficult to choose and evaluate. Finding volunteers for lifelong, or even five- to ten-year DR projects might be difficult if significant numbers were needed. The numbers, however, depend on the magnitude of the effect: if four out of six dedicated dieters, evading Makeham's coefficient, lived to >105–106 years of age, people might begin to take notice, though by that time other approaches to ageing control, by genetic manipulation, might have devalued DR as a clinical approach.

One can go on multiplying difficulties, and this, in fact, is what gerontologists have done so far. One must also suspect another and psychological factor, the fear of being thought overzealous in the style of the early rejuvenators. Weindruch and Walford, whose own research credentials are impeccable, confront the timid with reasoned argument and present here a research prospectus which is very difficult to fault or ignore. If we do not wilfully ignore it, we ought to be planning how to put it into effect rather than waste another 20 or so years arguing about it — landmark-

shifting has implications in developing a medicine of rate determination which goes considerably beyond the vulgar pursuit of mere longevity.

It is refreshing to come across a text that is not only readable and a comprehensive review of its subject, but also a challenge to researchers and funding bodies; we can either extend our control over the life cycle or prove the authors wrong by trial. Walford is a respected figure and the founder of gerontoimmunology who is known to carry out dietary trials on himself. Anyone who views that undertaking as eccentric should feel obliged to respond to the admirably presented case contained in this book. □

Alex Comfort, *The Windmill House, The Hill, Cranbrook, Kent TN7 3AH, UK, is Adjunct Professor at the Neuropsychiatric Institute, University of California at Los Angeles, and a former Head of the MRC Research Group on Ageing at University College London.*

## Down to Earth

Ian D. R. Mackinnon

**Meteorites and the Early Solar System.**

Edited by John F. Kerridge and Mildred Shapley Matthews. *University of Arizona Press: 1988. Pp. 1,269. \$55, £42.95.*

AT FIRST glance, the study of meteorites must appear to be an unusually obscure discipline, made more so by jargon and a confusion of terms. There have been successful introductory-level, undergraduate texts on the subject, but these have wisely glossed over the debates on interpretation of the meteoritic record. The detail required to do justice to such issues would hardly excite any publisher, and an aspiring author would risk losing tenure at the most tolerant of institutions. It has taken a unique combination to break the mould: an outstanding specialist publisher, and the patience and tenacity of two well-known members of the space-science community. Several excellent books, co-edited by Mildred Matthews, have appeared in the University of Arizona Press "Space Science" series; this most recent publication, co-edited with John Kerridge, maintains the standard.

In 1,200 pages, the editors and 68 collaborating authors make a stellar effort at providing a précis of early Solar System history interpreted through the meteoritic record. The broad sweep of meteorite studies, which cover many scientific disciplines and employ a multitude of techniques, is clearly evident in the tone and content of the volume. Moreover, it's not all petrography and isotopic signatures. Included are well-written summaries of current astrophysical models of pre-nebula and solar nebula evolution, as well as of

models of nebula accretion processes. In many of the contributions attempts have been made to relate models to the observational record and to present the pros and cons of contentious issues. Some of them, such as that linking asteroids and meteorites, are successful; others are less so, but as much because of gaps in the record than any lack of effort.

Although some may quibble with the precise positioning of chapters, or even with the emphasis on Solar System/nebula processes, there is no denying the success of the overall format. Telling questions to be asked of multi-authored volumes are how well concepts are related between different chapters, and whether authors who might not ordinarily be seen sipping tea together at the annual meeting of the minds have been persuaded to collaborate. Here it appears that contributors did bother to read drafts of other chapters, and have delivered the goods. In those chapters where unlikely collaborations have been imposed, good summaries of research have resulted and some have provided new insights which perhaps have surprised even the authors.

The book was in gestation for well over two years, and that time has been fruitfully used. Here we have a coherent and detailed overview of the dominant processes affecting chondrite meteorites, from early nebula formation (or survival?) of precursor grains, to their accretion and incorporation in an asteroidal parent body, followed by various impact processes, ejection from dominantly asteroidal orbits, and then eventual collection and transfer to the laboratory for study. Most chapters specifically address chondrite meteorites only, as these are believed to have undergone the least degree of modification since their formation about  $4.5 \times 10^9$  yr ago. However,

helpful explanatory references to achondrites such as SNCs (purportedly derived from Mars) and ureilites are scattered throughout. One chapter on igneous activity, which at first blush would be a questionable entry, has been neatly integrated into the rest of the text. The relationship between the terrestrial planets and asteroids, as revealed by meteorite studies, is also well developed in a series of chapters under the heading "Chemistry of Chondrites".

A firm editorial hand is also evident in the highly consistent use of symbols, acronyms, abbreviations and units of measure. In addition, the glossary and useful compilations of fundamental data in five appendices will be appreciated by many readers. The index has received the same thorough treatment — entries under "Meteorites, individual" occupy almost two pages and give a quick synopsis of the chondrites whose characteristics have primarily shaped current thinking on processes of the early Solar System.

In early correspondence on the proposed book, Kerridge made two important conditions for the final product. First, he felt that it needed to be well balanced and cohesive. Second, that it was not to be a vehicle for perpetuating dogma; many meteoriticists need reminding of this point, as it is often all too easy to follow the gurus. No doubt because of that prompting, many authors have made genuine efforts to sketch the nub of controversies where they exist and, more importantly, to suggest means for their eventual resolution. The final chapter on future directions is a thoughtful assessment of meteorite studies and a rich source of ideas for research proposals in this, and related, fields.

Meteoritics has come a long way since the days of Harvey Nininger and Harold Urey, to whose memory this volume is dedicated. Both were in their sunset years during the halcyon days of the Apollo programme, which gave new life to the study of meteorites as well as to that of the Moon. Both played a large part in the development of meteorite research and they would, no doubt, be proud of this singular community achievement. □

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• *Nature's* next review supplement, Spring Books, appears in the issue of 20 April. The books covered include *Digging into the Past*, Edwin Colbert's autobiography, *AIDS: The HIV Myth* by Jad Adams and *AIDS and Its Metaphors* by Susan Sontag, *Journey into Light*, a biography of C. V. Raman, *Does God Play Dice?* by Ian Stewart, Stuart Sutherland's dictionary of psychology, and two controversial works in archaeology. Among the reviewers are Jeremy Sabloff, Daniel S. Greenberg, Walter Gratz, Michael Berry, Roy Porter and Robert M. May.

## Learning of the Lucky Country

Robert A. Stafford

**Nature in Its Greatest Extent: Western Science in the Pacific.** Edited by Roy MacLeod and Philip F. Rehbock. *University of Hawaii Press: 1988. Pp.288. £27.20, \$34.*

**The Commonwealth of Science: ANZAAS and the Scientific Enterprise in Australasia, 1888–1988.** Edited by Roy MacLeod. *Oxford University Press: 1988. Pp.417. £25, \$45.*

THE motive proffered by Defoe's Captain Singleton for his peregrinations — "knowledge of things begat a desire of increasing it" — may serve as a simple explanation for the sustained European effort to penetrate, understand and organize the lands washed by the Pacific Ocean. Because Singleton was modelled on the circumnavigator Dampier, the analogy is particularly apposite, for Dampier's observations of natural phenomena created the vogue for South Seas exploration which endured throughout the eighteenth century and resulted in Britain's colonization of Australia.

With interest now turning to the Pacific basin as the theatre for the next major phase of economic development, it is appropriate that the historical role of Western science in the region should be assessed. Australia having celebrated its bicentenary, it is equally fitting that analysis of the place of science in the culture and economy of the island continent should be included in this analysis. The contributors to these two new books, which complement R. W. Home's *Australian Science in the Making* (Cambridge University Press, 1988), explore some of the more prominent features of what remains in many respects an intellectual *terra incognita*.

The function of the historian as pioneer is especially apparent in *Nature in Its Greatest Extent*. In conjunction with Philip Rehbock, Roy MacLeod has collected ten essays documenting European scientific activity in the Pacific from the eighteenth century to the present. The book opens many doors, revealing the wealth of materials that will enable future researchers to tell us in greater detail how Europe's discovery and exploitation of the lands of the Pacific transformed not only the region's peoples and habitats, but the mental landscape of Europeans. Individual contributions to the volume, however, are variable in quality, while the book as a whole is marred by incoherence of theme. The editors might have supplied a connecting overview in their introduction, but instead they pose questions

which the text fails to answer adequately.

The problem lies in defining the changing nature of the symbiotic relationship which has obtained between Western science and expansionism. Europeans were activated by greed as well as curiosity in coming to the Pacific. Science received patronage during the eras of exploration and settlement in exchange for the promise to deliver new resources for exploitation, new means of subduing hostile environments, effective methods for maintaining European ascendancy and cultural prestige for the metropolitan powers.

None of the essays covering the centuries before the Cold War sufficiently emphasizes that many European scientists were active agents of imperialism rather than mere fact-gatherers engaged in pushing back the frontier of the unknown. By means of their reports, maps, collections, taxonomic classifications and — more directly — their administrative and military duties, scientists facilitated the gradual imposition of European domination over the Pacific. As mediators of this new world, scientists played a large part in moulding European attitudes towards it. Their research agendas and results were, in turn, conditioned by European cultural norms, so that apparent objectivity often masked or reflected subjective metropolitan aspirations and anxieties. The same process was occurring in other regions which, as the editors fail to mention, presented challenges to Western intellectual assumptions and physical capabilities as daunting as those of the Pacific.

The book is divided into three sections. The first concentrates on the scientific content of the voyages of discovery and the tension between science and politics in their planning and execution. The second section, which offers more insightful analysis, examines how European science transplanted to the Australasian colonies sought to fulfil duties assigned by metropolitan savants while developing the impetus to assert its own authority and priorities. The long march of colonial scientists seeking intellectual independence was inexorably intertwined with the movement for political independence.

These essays likewise illustrate that science at the colonial periphery suffered severe constraints in comparison with that practised in Europe. Researchers were few on the ground, facilities rudimentary and funds scarce. Colonial culture often exhibited a philistine attitude towards the scientific enterprise, and the tyranny of distance between Port Moresby and Sydney was as real as that separating the South Pacific from London or Paris.

The final section moves into the twentieth century, when the winds of change which swept away the old empires cleared the ground for the new imperialism of the superpowers and the transnational corpo-

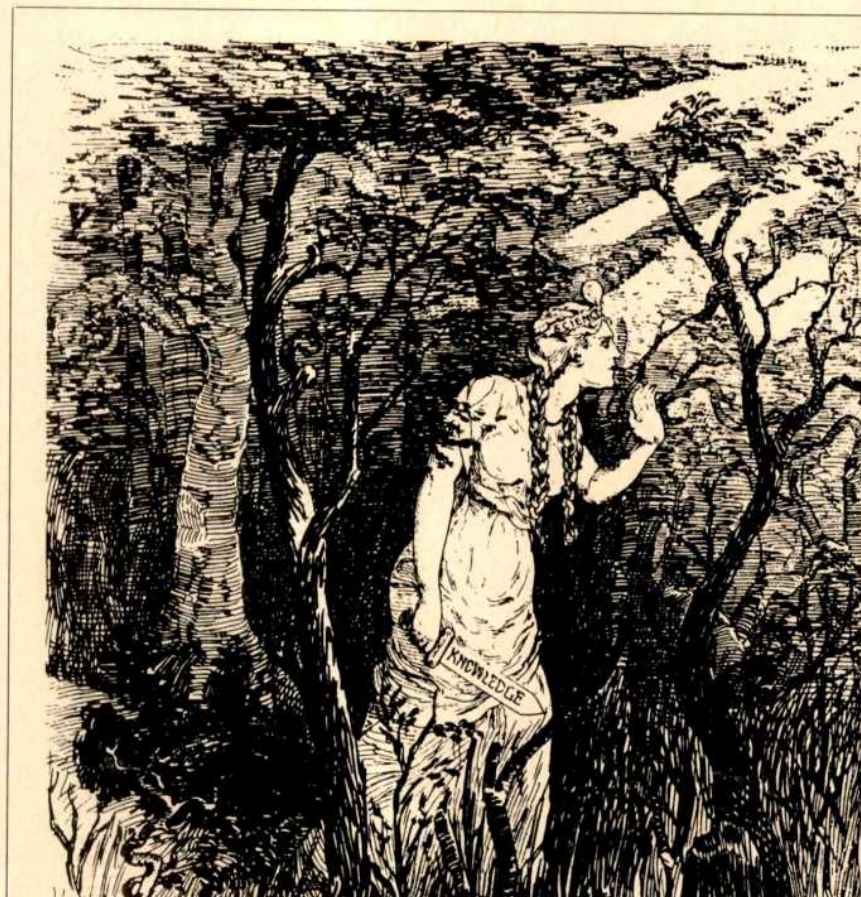


rations. In the interwar years, we learn, a burst of internationalism produced the Pacific Science Association, whose congresses have fostered an enduring tradition of cooperation among Pacific scientists. But following the Second World War, as many colonies discovered that political independence amounted to the perpetuation of subservience as client states of the United States or the Soviet Union, Pacific science traded old masters for new. The stars and stripes and the hammer and sickle now fly over far more scientific vessels or research stations than do the flags of other Pacific nations, or even the stubborn Tricolour.

As the last two chapters demonstrate, American and Soviet science remain as motivated by commercial and strategic concerns as were the scientific establishments which served the European powers during the exploration and colonization of the Pacific. But although the sciences have been employed for centuries as instruments of policy, scientists have with equal consistency shown themselves to be adept at exploiting governments to support research programmes that are ultimately driven by personal ambitions as well as disciplinary goals. It is in explicating the fine detail of this interplay that one of the most exciting frontiers of the history of science in its wider social dimension lies.

In *The Commonwealth of Science*, MacLeod embarks alone as editor to chart the history of science as it has developed in Australia during the twentieth century. On surer ground here than among the long Pacific swells, he builds upon the corpus of research published by the prolific Antipodean community of historians of science, as well as upon his earlier work on the British Association (the model for ANZAAS) and science policy throughout the empire. The story of ANZAAS, the Australian and New Zealand Association for the Advancement of Science, provides the prism through which we are invited to view science in the Antipodes from 1888 to the present. Coinciding with Australia's bicentenary, the ANZAAS centenary represents a convenient benchmark for surveying the evolving style and role of organized natural knowledge in the economic and cultural development of the nation.

The book contains several provocative essays, but again the quality is uneven. In two chapters discussing the foundation and early years of ANZAAS, MacLeod sets a standard of excellence — indeed, of eloquence — which some of his contributors fail to sustain. Institutional histories chronicling various disciplines alternate with analytical chapters which examine the meaning of science, the Australian national identity and man's place in nature. Rather than being a history of ANZAAS, the book provides an overview of the social role of science in Aus-



"The Pathfinder" — Science forging her way into the light (*Daily Telegraph*, 1911).

tralian history. This weakness is its strength, and in scope and penetration *The Commonwealth of Science* has a great deal to recommend it. We might wish, however, that MacLeod himself had completed the chronological narrative in the interests of keeping ANZAAS at the centre of things.

Nevertheless, we are left with an important contribution to the history of science in a former European colony and to the sociological literature on what it means to be Australian. Two recurring themes in the 15 chapters are the tensions between Antipodean colony and European metropolis, and between an imported culture and the alien environment upon which it has been imposed. Science figures squarely in these issues, for it has provided a venue for the articulation of Australian aspirations towards independence while simultaneously serving as a mediating device for the exploitation and understanding of a continent new to the European consciousness. Australia was born to the scientific age, so it is not surprising to learn that ANZAAS has functioned as a central reference point in the continuing adventure of creating an outpost of Western civilization in the South Pacific. It has proven a valuable public resource in a culture often branded as materialistic and anti-intellectual.

It remains to be seen whether Australia

will succeed in forging an effective national science policy. Such a strategy must harness the sciences to the goal of transforming Australia from the status of a supplier of primary products to that of a truly sovereign nation whose industrial and service sectors have attained critical mass and whose society has made the commitment to education and research-led change which is necessary for self-sustaining growth. As several contributors warn, the odds are at least even that Australians may fail, and that, as the Lucky Country devolves into a banana republic, the nation's tradition of scientific excellence will be dissipated like so many of her natural resources.

There are healthy signs, however, that this grim prognosis can and will be avoided by policy makers, and that Australia will maintain its reputation as the land of the second chance. The upsurge of interest in the role of science in the nation's history is one such encouraging sign, and these two books represent the cutting edge of the reappraisal. Anyone interested in the fate of Australian science, or indeed in the future of the country, should read *The Commonwealth of Science*. □

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## Divisions in botany

Deborah Charlesworth

**Mutation, Developmental Selection, and Plant Evolution.** By Edward J. Klekowski, Jr. Columbia University Press: 1988. Pp. 373. \$55.

EDWARD Klekowski has made a brave attempt to put together all the available information about mutation in higher plants. His aim is to use the data to support the argument that genetic loads in plants are increased, in comparison to those in animals, by somatic mutation.

The germ cells of higher plants are differentiated anew each reproductive season from several separate meristems, rather than being sequestered in a permanent germ line as in multicellular animals. This means that the number of mitotic cell divisions between the zygote stage and meiosis in the germ cells, which in a plant are formed by a line of cell descent, may on average be much higher than in, say, *Drosophila* or a female mammal (although the number is, of course, higher in male mammals). If there is some probability of a somatic mutation arising at each cell division, the frequency of mutations among the gametes produced by a tree increases with the number of mitoses, which in plants is correlated with age.

Evidence for a correlation between the number of cell divisions before meiosis and rate of mutation has recently been provided by Miyata's analysis of the rates of evolution of sex-linked loci in mammals, which have many more divisions in the male germ line than in the female (*Cold Spr. Harb. Symp. Quant. Biol.* **LII**, 863–867; 1987). Thus the mutation rate per generation should be higher in a long-lived plant, such as a tree, than in shorter-lived species. An old tree, or an old clone of a fern species, would also be expected to be chimaeric, with different meristems of the same genetic individual carrying different and independently generated mutations. In the book, a mass of anatomical and developmental detail on meristems illustrates the various patterns that might occur.

Klekowski devotes most of his text to evidence for these ideas. Unfortunately, it is difficult to estimate mutation rates and to compare species with different life spans, and the few data on mutation in plants are mostly from short-lived species. Because a species with characteristics that tend to increase the mutation rate would probably experience selection to reduce it, such estimates might in any case not show the expected differences. Klekowski therefore discusses mutational processes

and error correction mechanisms in great detail, but as so little is known about these in plants it is difficult to make the necessary comparisons. The frequency of mutant gametes in plants of different age, and the existence of chimaeras, could also be tested, but here again the data are too limited to be of much use.

In the absence of direct tests of the hypothesis, Klekowski marshalls many pieces of circumstantial evidence that agree with his expectations. This necessarily yields a somewhat confusing picture, as such arguments can be wrong in at least two ways. First, some of the phenomena may not be due to genetic load; for example, although genetic load could be the cause of low numbers of seeds per

ovule in trees, other explanations such as selection for high pollen production in these plants, are also possible. Second, interpretations other than Klekowski's could explain differences between the genetic loads of populations. Differences in breeding systems, for example, are largely ignored in the book.

Klekowski therefore does not make a watertight argument for his hypothesis. But, although his book is hard to read because of the diversity of the information presented (a glossary would have helped), it is certainly interesting and rich in ideas for future research. □

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## Vorsprung durch biotechnologie

Stephen Oliver

**Biotechnology Focus 1: Fundamentals, Applications, Information.** Edited by R.K. Finn and P. Präve. Hanser, Munich/Oxford University Press, New York: 1988. Pp. 436. DM128, \$85.

RIGHTLY or wrongly, English has become the international language of science. This is not to say that there is no need for scientific works to be written in other languages, but it does mean that translations into English must be viewed critically.

The American editor of *Biotechnology Focus* justifies the book's translation from German by asserting that in Europe, unlike the United States, genetic engineering has not excluded microbiology and chemical engineering from the area of biotechnology. I am sure there are those at, say, Madison or the Massachusetts Institute of Technology who would take issue with him. The other contention is that the book will unlock a literature unavailable to English-reading scientists. In fact, the principal biotechnologists in the German-speaking world habitually publish in English and less than 12 per cent of the references in *Biotechnology Focus* are to works in German. The longest chapter is on fungal pathogenicity, which is not an obviously biotechnological subject. This chapter is also one of those with the most German-language works in its bibliography; but it does not provide a ready entry to the German literature because only one of the 61 works (in all languages) quoted is actually referred to in the text. Clearly, editorial rigour has failed on one side of the Atlantic or the other, perhaps a case of passing the buck or *Verantwortung abschieben*.

*Biotechnology Focus* is described as a yearbook, but it is not in the mould of the

American Society of Microbiology's prestigious "Microbiology" series. Most of the contributions are of broad scope, such as the excellent chapter on scale-up theory by Hempel. This, however, sometimes makes them rather too general, as is the case with the contribution on computer control of antibiotic production. Sometimes a very general title disguises a very specific article — "Fundamental Methods of Genetics" mainly deals with the details of protoplast fusion technology using the hydrocarbon-metabolizing yeast, *Yarrowia lipolytica*. A great deal of experimental detail can be found in the book, for example in the account of cell immobilization. But this chapter appears in a section labelled "Applied Biotechnology", and so it is curious that the protocols described are for bench-scale work only. Overall, one has the impression that the editors did not have a clear idea of the book's purpose and so the authors have struggled manfully with an inadequate brief.

The topicality that might be expected of a yearbook is provided by an "Information" section. This is a good idea which is spoilt by the time-lag involved in translation — a company profile has Biogen still operating in Geneva, and the market values of bulk bioproducts are given in 1982 deutschmarks. The German-speaking world has made, and is making, an outstanding contribution to biotechnology; it is sad to see it so poorly represented. □

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• Two books published late last year will be useful to researchers in biotechnology who have an eye on the potential commercial value of their work. *Protecting Biotechnology Inventions: A Guide for Scientists* is by Roman Saliwanchik and is published by Science Tech Publishers, Madison, Wisconsin (distributed outside North America by Springer-Verlag); *Patents: A Basic Guide to Patenting in Biotechnology* is by R. S. Crespi and is published by Cambridge University Press.

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# Helium isotope ratios in circum-Pacific volcanic arcs

R. Poreda\* & H. Craig

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Volcanoes in the 'Ring of Fire' surrounding the Pacific Ocean are sited on tectonic arc segments marking the great subduction zones where oceanic crust returns to the mantle. Helium isotope ratios in volcanic gases along these arcs are close to those found in mid-ocean-ridge basalts, revealing the presence of primordial  $^3\text{He}$  released from the wedge of mantle material above the sinking plate. These results show that although the subduction of oceanic crust drives the arc volcanism, the subducted crust itself does not contribute a major fraction of the upwelling magma.

LAVAS associated with subduction, broadly termed arc volcanics, range in composition from basalt to dacite. Although a number of source regions and models have been suggested for the origin of arc magmas<sup>1-5</sup>, a unified model has been difficult

to construct because the isotopic and petrological signatures often require different source contributions. Craig *et al.*<sup>6</sup>, who measured  $^3\text{He}/^4\text{He}$  ratios in volcanic gases from Japan, the Marianas and Mt Lassen, showed that the dominant source of helium was the mantle wedge rather than subducted oceanic crust or sediment, both of which are rich in radiogenic  $^4\text{He}$ . We expand on that initial work to assess  $^3\text{He}/^4\text{He}$  ratios in all the North Pacific volcanic arc systems with respect to the role of the mantle wedge and the nature of the subducted crust in oceanic and continental arcs. Our most detailed study was in the Aleutian-Alaskan arc, a major feature erupting both tholeiitic and calc-alkaline lavas and grading from an oceanic arc on the west to a continental terrain in the east.

Helium isotope measurements were made on volcanic gases associated with recent arc volcanism. We sampled summit fumaroles whenever possible to minimize interaction of the gases with older crust enriched in radiogenic  $^4\text{He}$ . Helium in phenocrysts from recent subaerial or submarine lavas was analysed by crushing in vacuum<sup>7</sup>. Figure 1 shows the  $^3\text{He}/^4\text{He}$  ratios in the nine volcanic arc terrains studied: Table 1 lists these and other data for the individual volcanoes.

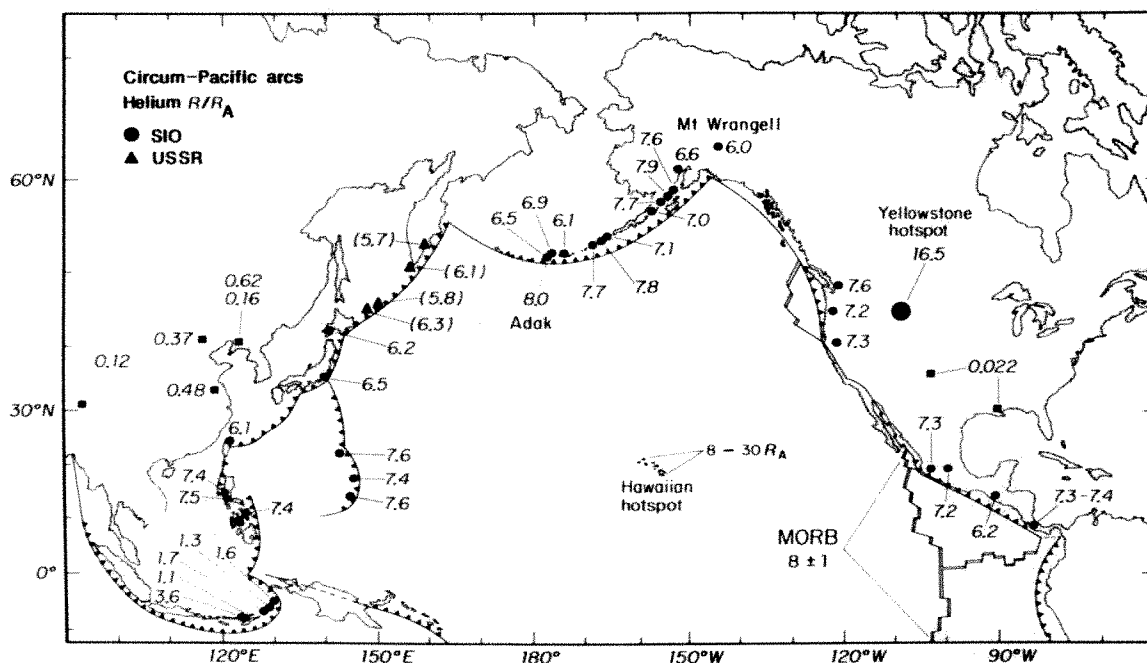


FIG. 1 Helium isotope ratios (relative to atmospheric He) in circum-Pacific volcanic gases and lavas, from this work (circles) and ref. 17 (triangles). A few data for the continental crust of China and the United States (squares) are shown for comparison, as are values for the Yellowstone and Hawaiian hotspots and for MORBs on the East Pacific Rise. Because radiogenic He has almost no  $^3\text{He}$  ( $R/R_A \approx 0.02$ ) the data show that the dominant source of helium in volcanic arcs is mantle (MORB) He with  $R/R_A = 8$ . Most arcs have He ratios from 7 to  $8 R_A$ , with a general mean of  $\sim 7.5 R_A$ , corresponding to  $X_M$ , the fraction of He derived from the mantle,  $\sim 7.5/8$ , that is, 94%. The Kurile-Honshu-Ryukyu arc system, including Japan, Taiwan, Kamchatka and the Kuriles, has a somewhat greater contribution of crustal or radiogenic

He, with a mean  $R \approx 6.4 R_A$  and  $X_M \approx 80\%$ . Similar values are found at Mts Spurr and Wrangell, volcanoes of the Aleutian-Alaskan arc system that are built on continental crust. The Banda arc, in the eastern sector of the Indonesian system, is caused by continental cratonic material (Australian plate) underthrusting oceanic crust. In this arc there is a much greater dilution of MORB He by the crustal component from the downgoing plate:  $R/R_A$  grades from 3.6 to  $\sim 1.4$ , proceeding eastward from the junction with the oceanic Sunda arc at Timor to the Banda Islands. The absolute flux of  $^3\text{He}$  from the mantle is known approximately<sup>25</sup>, so that volcanic arc fluxes of other volatiles can be derived from their ratios to  $^3\text{He}$  in arc lavas and gases.

### The Aleutian-Alaskan arc

Olivine phenocrysts from Mt Adagdak on Adak, and summit fumaroles on five volcanoes, Mts Okmok, Makushin, Griggs, Douglas and Augustine, have  $^3\text{He}/^4\text{He}$  ratios of  $R/R_A = 7.6$ – $8.0$  (where  $R_A$  is the  $^3\text{He}/^4\text{He}$  ratio in air), the highest volcanic arc values we have observed. These ratios are within the range for mid-ocean-ridge basalts ( $R/R_A = 8 \pm 1$ ; see, for example, refs 8 and 9) which agrees with the conclusion reached previously<sup>6</sup> that the mantle is the dominant source of helium in arc fluids. Along the entire length of this arc system from the Aleutian islands of Adak and Umnak, built on oceanic crust, to the Alaskan volcanoes Mt Douglas and Mt Augustine built on a

floor of Mesozoic sediments, there is no indication of any alteration in the  $^3\text{He}/^4\text{He}$  ratios from input of radiogenic  $^4\text{He}$ , even though trace-element and radiogenic isotope data show that sediments have contributed to these arc lavas<sup>4,10,11</sup>. At the eastern extremity of the arc, however, where the continental crust thickens and the trench-volcano gap abruptly widens<sup>12</sup>, the lower  $^3\text{He}/^4\text{He}$  ratios at Mts Spurr and Wrangell ( $6.6$  and  $6.0 R_A$ ) reflect significant contributions ( $\sim 20\%$ ) of crustal He from subducting terrigenous sediments<sup>12</sup> or the overriding continental plate.

Helium in volcanic gases consists of mantle He, radiogenic or 'crustal' He ( $R/R_A < 0.02$ ), and atmospheric He. The atmos-

TABLE 1 Helium isotope ratios in circum-Pacific volcanic arc samples

Sample number	Location	Sample type*	$R/R_A$	$\text{He}/\text{Ne}$ ( $\text{He}/\text{Ne}$ ) <sub>A</sub>	$R_C/R_A$ †	T (°C)	$\text{He}/\text{NC}\ddagger$ (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}$	Ref.
<b>ALEUTIAN ISLANDS</b>									
Adak Island									
AL-25-81	North coast	HS	6.09	11.3	6.58	24.0	51.7		
AL-30-81	Mt Adagdak	OL	7.94	100	8.01	[He] = $9.0 \times 10^{-9} \text{ cm}^3 \text{ g}^{-1}$		$0.7031 \pm 3$	32 <sup>4</sup>
Great Sitkin Island									
AL-26-81	Big Fox Creek	FF	3.95	2.2	6.49	97.0	14.0		
AL-27-81	Big Fox Creek	FF	6.86	193	6.89	97.6	—		
Atka Island: Kliuchef Volcano									
AL-34-81	Milky River	FF	6.00	30.2	6.15	96.0	36.0	$0.7033 \pm 1$	32, 4:
AL-35-81	Furn. field (615 m)	AS	5.22	17.4	5.40	93.0	38.0		
AL-37-81	Site A (410 m)	AS	5.06	34.8	5.17	97.0	59.0		
AL-38-81	Site B (410 m)	MP	5.20	61.0	5.26	84.0	82.3		
Umnak Island									
AL-17-81	Okmok volcano	GS	7.67	75	7.75	7.0	128	$0.7032 \pm 2$	10, 3:
AL-20-81	Geyser bight	HS	7.41	70	7.50	81.0	105		
Unalaska Island: Makushin volcano									
RM-82-MS	Summit	SF	7.83	1,500	7.83	96.0	250	$0.7031 \pm 2$	10, 3:
RM-80-MV2	Makushin valley	FF	6.60	110	6.65	—	134		
RM-83-57	Field No. 7	FF	5.91	300	5.93	—	131		
RM-82-WF	West flank	FF	5.04	70	5.10	—	78		
RM-80-MV1	Makushin valley	FF	4.91	37	5.01	98.0	66		
AL-3-81	Makushin valley	FF	4.97	94	5.01	97.2	105		
AL-6-81	Glacier valley	FF	4.52	53	4.58	97.0	58		
RM-82-GV1	Glacier valley	FF	4.04	11.4	4.30	152	53		
AL-5-81	Glacier valley	AS	3.80	24	3.91	92.0	40		
RM-83-77	Test well	W	3.68	41	3.74	193	306		
RM-83-41	Chloride spring	HS	1.27	1.5	1.60	—	3.1		
Akutan Island									
AL-9-81	East flank	AS	7.06	124	7.10	86.6	157	$0.7032 \pm 2$	10, 3:
AL-11-81	Hot Spring bay	HS	5.23	4.8	6.10	84.5	14.9		
<b>ALASKAN PENINSULA</b>									
AL-24-81	Gas rocks	S	6.91	114	6.95	26.0	6,140		
K-78-G9	Mt Griggs	SF	7.49	34.8	7.66	96.0	—		
RM-82-DG	Mt Douglas	SF	7.89	850	7.89	93.5	—		
78-A-80G	Augustine volcano	SF	5.90	3.8	7.66	97.2	38.3		
RM-82-AG4	Augustine volcano	SF	7.55	135	7.60	120	—		
RM-82-SPR	Mt Spurr	SF	6.40	24.0	6.62	94.1	—	$0.7037 \pm 3\%$	
RM-82-WR	Mt Wrangell	SF	5.30	6.1	6.05	—	—	$0.7035 \pm 3\%$	
AL-113	Mt Wrangell	SF	6.02	400	6.03	150	1,790		
<b>CASCADES</b>									
	Mt Baker	SF	7.61	900	7.62	—	3,416		
	Mt Hood	SF	7.17	112	7.20	—	2,015	$0.7035 \pm 3$	42, 4:
L-2(SW)	Lassen park	AS	7.29	3,000	7.29	87	710	$0.7036 \pm 3$	42, 4:
L-8(BH)	Lassen park	AS	7.06	520	7.08	90	—		

The  $^3\text{He}/^4\text{He}$  measurements were made on a Clarke-type double-collecting mass spectrometer (GAD) as described previously<sup>7-9</sup>. In all cases helium was separated from neon on activated charcoal at 37 K to eliminate effects of variable He/Ne ratios on the measured  $^3\text{He}/^4\text{He}$  ratios of samples and standards. A secondary standard (Yellowstone Park gas:  $R/R_A = 16.45$ ,  $\text{He}/\text{Ne} > 1,000$ ) was used. Neon was also measured and the He/Ne ratios (column 5) were used to correct the measured  $^3\text{He}/^4\text{He}$  ratios, assuming all Ne to be atmospheric. Precision of the  $^3\text{He}/^4\text{He}$  ratios is 1%, unless otherwise stated. Total gas chemistry and  $^{13}\text{C}$  data will be reported elsewhere.

\* Sample key: SF, Summit fumarole; FF, flank fumarole; AS, acid spring; MP, mudpot; HS, hot spring; GS, gas seep; W, well; OL, olivine; CPX, pyroxene; F, fumarole. Data for OL and CPX examples include, at column 7, the He concentration in the phenocrysts, in  $10^{-9} \text{ cm}^3 \text{ STP g}^{-1}$ .

†  $R_C$  is the  $^3\text{He}/^4\text{He}$  ratio corrected for atmospheric He contamination<sup>10</sup>:  $R_C = (RN - R_A N_A)/(N - N_A)$ , where  $R$  is the  $^3\text{He}/^4\text{He}$  ratio,  $N = \text{He}/\text{Ne}$ , and  $A$  denotes the 'atmospheric' ratios.  $N_A$  is taken as the He/Ne ratio in air (0.288) for most fumaroles, or in water solution at 10 °C (0.230) for most hot-spring samples.

pheric component (obtained from the  $\text{He}/\text{Ne}$  ratio<sup>6</sup>) includes both entrained air in the natural feature and air added as a sampling artefact. The extensive data on Makushin volcano fumaroles show that the atmospheric and radiogenic components are correlated (see Table 1), indicating that the atmospheric component has for the most part been added in the crustal environment which contributed the radiogenic component.

### Western North America

Three volcanoes of the Cascades have  $^3\text{He}/^4\text{He}$  ratios of 7.2–7.6  $R_A$  in summit fumaroles and acid springs. Mts Baker, Hood and Lassen represent the northern, central and southern points

of the High Cascades respectively, but whereas the first two are andesitic volcanoes, Lassen is a dacite dome. Thus there is no evidence for a correlation of  $^3\text{He}/^4\text{He}$  ratios with the degree of magmatic differentiation in these volcanoes.

In the Trans-Mexican volcanic belt, two high-temperature geothermal systems, Los Azufres and La Primavera, have similar ratios,  $R = 7.3 R_A$ . Following the Middle American arc into Guatemala, the Zunil field associated with Santiaguito volcano<sup>13</sup> has fumaroles with distinctly lower values of  $R = 6.2 R_A$  that are boiling off from a deep chloride-rich water resident in older country rock, so that the  $^3\text{He}/^4\text{He}$  ratio is probably a lower limit. In Costa Rica, a summit fumarole on Poas volcano and

TABLE 1 (continued) Helium isotope ratios in circum-Pacific volcanic arc samples

Sample number	Location	Sample type*	$R/R_A$	$\text{He}/\text{Ne}$ ( $\text{He}/\text{Ne}$ ) <sub>A</sub>	$R_C/R_A$ †	T (°C)	He/NC‡ (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}$	Ref.
MEXICO									
	La Primavera	W	7.34	2,500	7.34	—	1,636	0.7039 ± 3	44
	La Primavera	F	7.16	55	7.26	95.0	184		
	Los Azufres	W	7.21	—	—	—	—		
GUATEMALA									
GM-1-80	Zunil field	AS	5.95	21.1	6.18	88.0	—	0.7039 ± 1	45, 46
GM-3-80	Zunil field	AS	6.01	—	—	25.0	259		
GM-8-80	Zunil field	W	4.19	>100	4.22	96.0	17.9		
	Pacaya volcano	OL	6.8 ± 1.5	—	—	[He] = 4.8 × 10 <sup>-8</sup> cm <sup>3</sup> g <sup>-1</sup>		0.7039	45, 46
COSTA RICA									
	Poas volcano	SF	6.84	14.4	7.20	315	145	0.7038 ± 1	47
85-3130	Marivalles	W	7.20	36	7.38	—	177		
85-3131	Marivalles	W	6.43	90	6.49	—	337		
85-3132	Marivalles	W	7.33	160	7.37	—	725		
85-3136	Marivalles	W	6.97	72	7.05	—	390		
MARIANAS									
AG-1	Agrigan volcano	HS	6.92	10.6	7.42	46.0	—		
TT192D30¶	Eifuku seamount	OL	6.66	—	7.65	[He] = 1.4 × 10 <sup>-9</sup> cm <sup>3</sup> g <sup>-1</sup>		0.7036	
TT192D01¶	Ruby seamount	OL	7.06	—	7.62	[He] = 3.4 × 10 <sup>-9</sup> cm <sup>3</sup> g <sup>-1</sup>		0.7035 ± 1	
	Ruby seamount	CPX	6.90	—	7.22	[He] = 6.4 × 10 <sup>-9</sup> cm <sup>3</sup> g <sup>-1</sup>			
PHILIPPINES									
PHIL-13	Mt Pinatubo	SF	7.43	500	7.44	97.5	402	0.7044	16
PHIL-19	Biliran Island	SF	7.41	960	7.42	98.0	205		
PHIL-21	Taal volcano	SF	7.45	100	7.50	99.0	476	0.7048	16
TAIWAN: TATUNG									
SHP-1	Mud volcano	MP	6.14	350	6.15	97.0	—	0.7040 ± 2	48
THC-2	Acid spring	AS	5.40	100	5.44	—	—		
DYK-1	Hell Valley	AS	4.40	5.7	5.00	96.0	41.1		
SCC-1	West End	AS	4.64	127	4.66	82.0	—		
CS-2	Chinshan	AS	3.93	4.5	4.77	72.0	11.9		
MTS-1	Central region	AS	4.35	950	4.35	92.0	680		
JAPAN									
CW-1	Hakone volcano	W	6.50	140	6.54	172	135		
H-6	Hakone volcano	W	6.62	93	6.68	137	261		
H-9	Hakone volcano	W	5.99	18.2	6.28	144	161		
USU-2	Usu volcano	SF	6.15	236	6.16	736	—	0.7040	38
USU-3	Usu volcano	SF	6.07	125	6.10	236	—		
USU-15	Usu volcano	SF	6.19	107	6.23	666	—		
INDONESIA									
	Serua	SF	1.27	1.7	1.64	102	11.7	0.7084 ± 7	23
	Teon	SF	1.31	130	1.31	99	990	0.7076 ± 1	23
	Damar (400 m)	FF	1.69	600	1.69	135	2,630	0.7065 ± 1	23
	Damar (700 m)	SF	1.65	3,200	1.65	170	2,150		
	Sirung	SF	1.03	1.7	1.07	95	9.8		
	Lewotolo	SF	3.62	500	3.62	490	700	0.7056*	
	Lewotolo	SF	3.57	1,180	3.57	490	990		

The  $\text{N}_2/\text{Ar}$  ratio can often be used to estimate the proper correction factor when the correction is important. When a range is shown for  $R_c$ , the upper and lower limits are calculated from the air and solubility correction factors respectively.

‡ NC, Non-condensable gases.

§ C. J. Nye (personal communication).

|| Re-analyses of original gas samples from Craig *et al.*<sup>6</sup> with Ne separation from samples and standards.  $R/R_A$  values are lower when Ne is removed from air standards: for example, the two Lassen Park analyses given here are 9% lower than the earlier values<sup>6</sup> measured without Ne removal.

¶ Submarine basalts dredged by Stern from Mariana arc seamounts: Eifuku at 21°45' N, 144°10' E, and Ruby at 15°12' N, 45°25' E on NSF-supported expedition: he also supplied the Sr isotope data.

\* J. C. Varekamp (personal communication).



borehole gases from the Marivalles geothermal field continue the consistency in  $^3\text{He}/^4\text{He}$  ratio ( $7.4 R_A$ ) for North American arc terrains.

### Western Pacific arcs

Helium isotope ratios in the central Western Pacific arcs, the Marianas and Philippines, are very similar to the North American values. In the Mariana arc a hot spring on Agrigan volcano<sup>6</sup> has a  $^3\text{He}/^4\text{He}$  ratio of  $7.4 R_A$ , and phenocrysts from two submarine volcanoes<sup>14</sup> have ratios of  $7.2$ – $7.65 R_A$ . Volcanic gases from the Philippine arc have similar He signatures:  $^3\text{He}/^4\text{He}$  ratios from Taal, Mt Pinatubo and Biliran Island range from  $7.4$  to  $7.5 R_A$ , identical to the Mariana and North American values. Lead isotope ratios in these volcanics also reflect a minimum contribution of subducted sediment<sup>15</sup>, although Sr isotope ratios are much higher than in western North American arcs<sup>16</sup>.

In the northwestern Pacific arcs, the Kurile–Honshu–Ryukyu system, a clear difference in the volcanic arc He signature is evident. In Japan, steam wells from the inner caldera of Hakone volcano have  $^3\text{He}/^4\text{He}$  ratios of  $6.5 R_A$ , and high-temperature ( $700^\circ\text{C}$ ) fumaroles on Usu volcano in Hokkaido have similar ratios of  $6.2 R_A$ . These Japanese ratios are distinctly lower than the Aleutian and North American values, but very similar to values in the Kuriles and Kamchatka<sup>17</sup>, shown in Fig. 1. Recent work<sup>18,19</sup> on Japanese hot-spring and volcanic gases shows a wide range in  $^3\text{He}/^4\text{He}$  ratios, converging in the high-ratio, high-He samples to a statistical distribution with a mean  $R/R_A = 6.5 \pm 1.5$ , uncorrelated with He or air content. It seems likely that this range of He ratios simply represents analytical scatter about a mean volcanic ratio,  $R/R_A = 6.5$ , which agrees well with both our earlier<sup>6</sup> and present data.

In northern Taiwan, gases from acid hot springs associated with Pleistocene volcanism in the Tatung region<sup>20</sup> range in  $^3\text{He}/^4\text{He}$  ratio from  $4.4$  to  $6.1 R_A$ . The Tatung field lies near the intersection of the Ryukyu and Luzon arcs,  $\sim 150$  km above the Benioff Zone defined by the subduction of the Philippine Plate beneath Eurasia<sup>21</sup>. Although the range in  $^3\text{He}/^4\text{He}$  ratios indicates the presence of a radiogenic  $^4\text{He}$  component, the highest ratio measured,  $6.1 R_A$ , is very consistent with the low  $^3\text{He}/^4\text{He}$  ratios in the Kurile and Japanese volcanoes.

### Indonesia

The most striking contrast to the relatively uniform  $^3\text{He}/^4\text{He}$  ratios observed in the other arc terrains ( $6$ – $8 R_A$ ) is the isotopic composition of He in the active volcanoes of the Banda arc in the eastern sector of Indonesia. The summit fumarole at Lewotolo, on the western edge of the Banda sector, has He with  $R/R_A = 3.6$ : eastward along this arc, three volcanoes have  $^3\text{He}/^4\text{He}$  ratios as low as  $R/R_A = 1.1$ – $1.6$ . Although it is possible that addition of  $^4\text{He}$  from circulating geothermal fluids is significant, it appears remote at Lewotolo and Damar ( $1.7 R_A$ ) with summit fumaroles at  $490^\circ\text{C}$  and  $170^\circ\text{C}$  respectively. In two cases, Serua and Sirung, the correction for atmospheric helium is large, but the corrected ratios also point to a helium source for all Banda arc volcanoes that is much more radiogenic than in any other arc gases so far analysed. The Banda arc lavas show extreme enrichments in Sr, Nd and O isotope ratios, all suggesting a major input of crustal material<sup>22–24</sup>.

### Discussion

The data here show that our previous observation<sup>6</sup> that active transport of mantle gases occurs in arc regimes is a general phenomenon in major volcanic arcs. In summit fumaroles uncontaminated by radiogenic  $^4\text{He}$  from older country rock, the  $^3\text{He}/^4\text{He}$  ratio is very close to that of mid-ocean-ridge basalts (MORBs) ( $R/R_A = 8$ ). Except for the Banda arc, the result of a unique arc–continent collision, the uniformity in  $^3\text{He}/^4\text{He}$  ratios contrasts markedly with the differences in tectonic setting and lava compositions of the arc systems. These range from the

continental arcs of western North America, where young crust is subducting into sediment-filled trenches, to the oceanic arcs of the Western Pacific, where old crust subducts into nearly sediment-free trenches. Andesites and dacites dominate the continental arcs whereas basalt and basaltic andesite are the major magma types in the oceanic arcs.

The arc helium signatures ( $R/R_A = 6$ – $8$ ) point to a MORB source dominating the production of arc lavas. Craig *et al.*<sup>6</sup> observed that if arc volcanics were simply remelted downgoing oceanic crust, then very low  $^3\text{He}/^4\text{He}$  ratios,  $\sim 2 R_A$  or less, would be observed because of radiogenic  $^4\text{He}$  production in old oceanic crust which should retain little if any primordial  $^3\text{He}$  (ref. 8). Hydrothermal circulation also removes large quantities of  $^3\text{He}$  from the oceanic crust. With a degassing rate of  $4 \text{ atoms cm}^{-2} \text{ s}^{-1}$  ( $1,000 \text{ mol yr}^{-1}$ ) of  $^3\text{He}$  (ref. 25) and a formation rate of  $3 \text{ km}^2 \text{ yr}^{-1}$  of oceanic crust, hydrothermal circulation will remove  $>10^{-5} \text{ cm}^3 \text{ s}^{-1}$  of helium to a depth of 5 km (ref. 8). Partitioning studies<sup>26</sup> show that  $<1\%$  of helium in a basalt lava resides in phenocrysts, so that cumulate gabbros do not hold  $^3\text{He}$ . Direct measurement<sup>27</sup> of 100-Myr-old basalt glass shows low  $^3\text{He}$  concentrations ( $0.5$ – $0.2 \times 10^{-12} \text{ cm}^3 \text{ g}^{-1}$ ) with ratios  $<3 R_A$ , because of radiogenic He production and diffusive losses<sup>9</sup>. These considerations all point to the conclusion that old oceanic crust is low in  $^3\text{He}$ , has a  $^3\text{He}/^4\text{He}$  ratio dominated by radiogenic  $^4\text{He}$ , and cannot be the major source of helium in arc lavas.

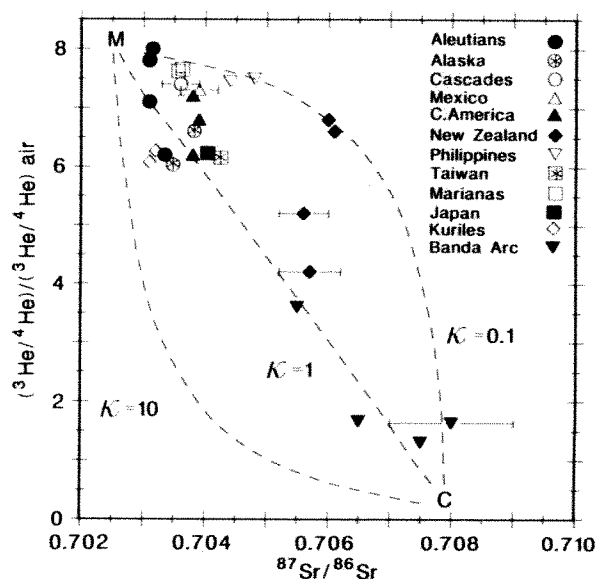


FIG. 2 A plot of  $^3\text{He}/^4\text{He}$  against  $^{87}\text{Sr}/^{86}\text{Sr}$  for 12 circum-Pacific arcs. The  $^3\text{He}/^4\text{He}$  data are from this paper, refs 17 and 50 (Kuriles), and ref. 36 (SIO data) and ref. 49 (New Zealand).  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios for lavas from the same volcano as the helium data were used whenever possible; error bars on Sr ratios reflect the range measured at a volcano. The curves are mixing trajectories between a MORB component ('M' =  $8 R_A$ ,  $0.7025$ ) and a crustal/sedimentary end member ('C' =  $0.2 R_A$ ,  $0.7080$ ) defined by a parameter  $\kappa = [(^{87}\text{Sr}/^{86}\text{Sr})_{\text{CRUST}} / (^{87}\text{Sr}/^{86}\text{Sr})_{\text{MORB}}]$ . Sedimentary  $^{87}\text{Sr}/^{86}\text{Sr}$  values are from refs 51 and 52. Note that whereas the  $^3\text{He}/^4\text{He}$  ratio of component 'C' is fixed at  $\sim 0$  (radiogenic He), the  $^{87}\text{Sr}/^{86}\text{Sr}$  will vary for different sources (for example,  $0.720$  for old Precambrian crust), so that the curves for  $\kappa$  values are pinned at the 'M' composition, but move left or right for 'C' components with smaller or larger  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. Thus the  $\kappa$  values for mixtures depend directly on the Sr isotope ratio assumed for 'C'. The He/Sr ratio of MORB is reasonably well constrained within a factor of two ( $^4\text{He} \approx 10^{-5} \text{ cm}^3 \text{ g}^{-1}$ ,  $\text{Sr} \approx 120 \text{ p.p.m.}$ ,  $\text{He}/\text{Sr} \approx 3.2 \times 10^{-4}$  molar ratio). However, values of  $\kappa$  could vary by a factor of 100, from a minimum  $\kappa \approx 0.1$  for pelagic sediments with very low He retentivity, to  $\sim 0.2$  for old basaltic crust which lost all initial He at eruption, to  $\kappa \approx 10$  for Mesozoic and older continental crust ( $2.7 \text{ p.p.m. U}$ ,  $370 \text{ p.p.m. Sr}$ ). Thus an average 200-Myr-old crust which retained 50% of its radiogenic He might have a He/Sr ratio twice that of MORB.

Stern<sup>28</sup> discussed the similarities in  $^{87}\text{Sr}/^{86}\text{Sr}$ ,  $\text{K}/\text{Rb}$  and  $\text{K}/\text{Ba}$  ratios in primitive arc volcanics and oceanic island basalts and suggested that an enriched source region produces arc lavas. However, we note that *no arc terrain has a  $^3\text{He}/^4\text{He}$  ratio higher than that of normal mid-ocean-ridge basalt*. The  $^3\text{He}/^4\text{He}$  ratios of oceanic islands fall into two groups relative to MORB helium<sup>29</sup>: 'high- $^3\text{He}$ ' hotspots (for example Hawaii, Iceland, Samoa) have ratios significantly higher than MORBs<sup>9,29</sup> with  $R/R_A \approx 11$ –30, and could not be a source for arc magmas. However, the 'low- $^3\text{He}$ ' hotspots with  $R/R_A \approx 5$ –8 (for example, Jan Mayen, Azores or Gough<sup>30</sup>), do resemble arc magmas in their He signatures, and ratios of  $^3\text{He}/^4\text{He}$  in fumaroles and of  $^{87}\text{Sr}/^{86}\text{Sr}$  in lavas of the Aleutians and Cascades overlap the fields for Mohs Ridge and Azores Platform basalts<sup>31–34</sup>. As with other geochemical tracers the relationships between  $^3\text{He}/^4\text{He}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  do not provide a unique solution to the source for arc lavas, but it seems improbable that 'low- $^3\text{He}$ ' mantle source regions, principally found in the Atlantic Ocean<sup>29</sup>, should exist beneath every circum-Pacific volcanic arc.

The  $^3\text{He}/^4\text{He}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  distribution can also be produced by mixing a component from the subducting slab, rich in  $^{87}\text{Sr}$  and  $^4\text{He}$ , with a normal MORB mantle (He and Sr ratios of  $8.0$ – $8.5 R_A$  and  $0.7024$ – $0.7028$  respectively). Because of the similarity in  $^3\text{He}/^4\text{He}$  ratios between arc and MORB helium, it seems more probable that the source for arc volcanism resembles the MORB source in helium and that differences in  $^3\text{He}/^4\text{He}$  ratios and other geochemical tracers such as  $^{87}\text{Sr}/^{86}\text{Sr}$  result from a variable slab contribution from sediments or altered oceanic crusts.

### Helium–strontium relationships

Figure 2 shows the  $^3\text{He}/^4\text{He}$  data and measured or estimated  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios for lavas from the same sites (Table 1) on ten circum-Pacific arcs, with trajectories calculated for mixing a MORB component (M) with a possible crustal or sedimentary source (C). Most of the data are consistent with the addition of up to  $\sim 25\%$  of crustal He ( $R/R_A = 8$ – $6$ ), and up to  $\sim 65\%$  of the crustal Sr component, to a MORB source. That is, the values cluster in the region for  $\kappa < 1$  (see Fig. 2 legend), so that the fraction of mantle He in arc lavas is equal to or greater than the fraction of mantle Sr, consistent with a lower He/Sr ratio in the crustal component. The 'mixing curves' are of course subject to considerable uncertainties inherent in representing two very different elements such as He and Sr. Fumaroles on two andesitic volcanoes (Ngauruhoe,  $516^\circ\text{C}$ ; White Island,  $540^\circ\text{C}$ ) in New Zealand<sup>36</sup>, and two volcanoes (Pinatubo and Taal) on the West Philippine (Manila) arc, lie on the minimum  $\kappa$  curve ( $\kappa = 0.1$ ), suggestive of a MORB source diluted with a low He/Sr crustal component, possibly subducted pelagic sediment with a high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio and Sr content.

Contamination of arc magmas during ascent through continental crust does not seem to have a large effect on  $^3\text{He}/^4\text{He}$  ratios. Because the U/Sr ratio is higher in crustal rocks than in MORB, pre-Cenozoic rocks that retain helium should have He/Sr ratios greater than MORB ( $\kappa > 1$ ) and on mixing with arc magmas should produce large changes in  $^3\text{He}/^4\text{He}$  ratios relative to  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. In the Alaskan arc (Fig. 1)  $^3\text{He}/^4\text{He}$  ratios in the continental volcanoes of Mts Griggs, Douglas and Augustine are not lower than those of summit fumaroles in the Aleutian arc: indeed Mt Douglas ( $R = 7.9 R_A$ ) has the highest  $^3\text{He}/^4\text{He}$  ratio in the arc. Thus the influence of the Mesozoic continental crust on the  $^3\text{He}/^4\text{He}$  ratios of these magmas seems to be less than a 5% dilution. Sr isotope ratios support this conclusion, as no systematic gradient exists from the Aleutian to the Alaskan sectors<sup>32,35</sup>. However,  $^3\text{He}/^4\text{He}$  ratios for the two easternmost volcanoes in the chain, Mt Spurr ( $6.6 R_A$ ) and Mt Wrangell ( $6.1 R_A$ ), are significantly lower (Fig. 2, on the  $\kappa \approx 1$  mixing curve), very probably because of dilution with helium from a thicker continental crust.

The Banda arc data lie at the opposite extreme in Fig. 2, with

a crustal component amounting to  $\sim 80\%$  for He ( $R/R_A = 1.6$ ), and  $\sim 80$ – $90\%$  for Sr based on a  $0.7080$  end member. If, however, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of the crustal end member is closer to  $0.720$  (refs 23, 24), as expected for the Precambrian Australian craton, the data would require  $\kappa \approx 5$  for a mixing curve, corresponding to a helium-rich crustal component. In fact the He/Sr ratio in Precambrian rocks is probably at least ten times the MORB ratio, and this end member would also fit the correlation between  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$ , which suggests a 25% sialic contribution<sup>23,24</sup>. Conversely, the Sunda arc lavas in the western Indonesian sector, with Sr ratios<sup>23</sup> of  $\sim 0.7048 \pm 0.0004$  derived from subduction of oceanic crust, are predicted (Fig. 2) to have  $^3\text{He}/^4\text{He}$  ratios of  $R/R_A = 7 \pm 0.5$ , with a well defined transition to the much lower Banda arc ratios near Lewotolo ( $R/R_A = 3.6$ ).

### Regional variations

Considering the  $^3\text{He}/^4\text{He}$  ratios in summit fumaroles, phenocrysts and least radiogenic samples, six volcanic arcs in Fig. 1 (the Aleutian–Alaskan arc, Cascades, Trans-Mexican belt and the Central American, Mariana and Philippine (east and west) arcs), have essentially indistinguishable mean ratios with  $R/R_A \approx 7.5 \pm 0.5$ , corresponding to a mantle He fraction  $X_M \approx 94 \pm 6\%$ . In the North Pacific only the Kurile–Honshu–Ryukyu arc system, including Kamchatka and the Kuriles, Japan and Taiwan, has clearly lower He ratios, with a mean value of  $R/R_A \approx 6.4$ , and a mantle He fraction of  $\sim 80\%$ . Although each of these arcs has a distinct geochemical signature which may reflect the composition of the mantle wedge or the age and composition of the overriding or subducting crust, no systematic correlation between these variables and the  $^3\text{He}/^4\text{He}$  ratios exists. Thus the Mariana arc, where the oldest oceanic crust (Jurassic) is subducting, has among the highest He ratios,  $R/R_A = 7.6$ , like the Philippine and Aleutian arcs where considerably younger crust is subducted. Most of the data points in Fig. 2 require a helium-poor component with a low He/Sr ratio ( $\kappa \approx 0.4$ ), either sediment or altered oceanic crust, mixing with a MORB source. Radiogenic production of  $^4\text{He}$  during 100 Myr with  $0.1$  p.p.m. of U should produce a He/Sr ratio of  $\sim 0.2$  times the MORB ratio if no He is removed, but because of the mobility of He and the lack of data on crustal He/Sr ratios, it is difficult to predict the nature of the 'C' component in individual cases.

Helium in arcs associated with subducting oceanic crust is almost entirely derived from a dominant MORB component in the mantle wedge, but we do not know at present whether the subducting crust is almost totally depleted of both  $^3\text{He}$  and  $^4\text{He}$ , or whether radiogenic He is still present in significant amounts but is not mobile. In the Banda arc of Indonesia most of the volcanic arc He (80%) is derived from subducting continental crust associated with a Precambrian craton, so that here at least a substantial increment of  $^4\text{He}$  relative to the He derived from the mantle wedge is retained during subduction and then mobilized into the erupting arc magma. Despite our uncertainties as to the processes actually responsible for cooking the final  $^3\text{He}/^4\text{He}$  recipes for arc volcanic gases and lavas,  $^3\text{He}$ , because it has essentially zero concentration in the oceanic and continental crust, is the most powerful tracer we have for studying the crustal contribution to arc magmas. □

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# The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase

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The maternal gene *torso*, required for determination of anterior and posterior terminal structures in the *Drosophila* embryo, was cloned using P-element tagging. Genetic evidence suggests that the action of the gene product is spatially restricted to the terminal regions; the *torso* messenger RNA, however, is evenly distributed. Structural similarities of the predicted *torso* protein with growth-factor receptor tyrosine kinases suggest that the spatial restriction of *torso* activity results from a localized activation of the *torso* protein at the anterior and posterior egg pole.

EARLY pattern formation in the *Drosophila* embryo depends upon maternal gene products provided during oogenesis and deposited in the freshly laid egg. For the determination of the anteroposterior axis of the embryo three classes of maternal genes have been identified: the anterior, the posterior and the terminal class<sup>1</sup>. Each class defines a pattern-forming process which results in the localization and/or activation of at least one maternal gene product which is necessary for the appropriate spatial expression of the zygotic target genes. In addition to the anterior and posterior genes, which function through localized components, a third organizing activity is required for the formation of the unsegmented terminal regions. At least six maternally expressed genes are necessary for the formation of the most anterior and posterior regions of the larva, acron and telson (as defined in ref. 1): *torso*(*tor*)<sup>2</sup>, *trunk*(*trk*)<sup>2</sup>, *torso*like(*tsl*) (H.-G. Frohnhofer, personal communication), *f(1)Nasrat* (*fs(1)N*)<sup>3</sup>, *fs(1)polehole* (*fs(1)ph*)<sup>4</sup> and *l(1)polehole* (*l(1)ph*)<sup>5</sup>. Embryos derived from mothers homozygous for mutations in this terminal class of genes fail to develop the labrum and the head skeleton is reduced in size. In addition, all elements posterior to the seventh abdominal segment are absent, including

the eighth abdominal segment and the telson. Mutations in any gene of the terminal class give an identical phenotype, which suggests that these genes are part of a single pattern-forming process that results in the spatially restricted expression or in the activation of zygotic terminal genes such as *tailless*(*tll*)<sup>6,7</sup>. In this pathway *torso* appears to play a crucial part, which is suggested by the phenotype of *torso* gain-of-function alleles. These mutations produce a phenotype opposite to the loss-of-function alleles<sup>7</sup>: elements of the termini are formed but the segmentation of the thorax and abdomen is suppressed. However, segmentation is restored in embryos which are in addition mutant for a null allele of *tll*. This indicates that the function of the *torso* product is mediated via *tll*, and suggests that in embryos of *torso* gain-of-function alleles, *tll* is expressed everywhere as a result of an ectopically active *torso* product. In the wild-type embryo the locally active *torso* product therefore provides cues required for correct spatial activity of terminal zygotic genes such as *tll*. Because cytoplasmic transplantation experiments revealed no localization of wild-type *torso* activity, it has been suggested<sup>7</sup> that the spatial restriction of *torso* activity may arise from a localized activation of the *torso* product through a modification mechanism by a preexisting spatial signal, which might be provided by one of the other terminal genes.

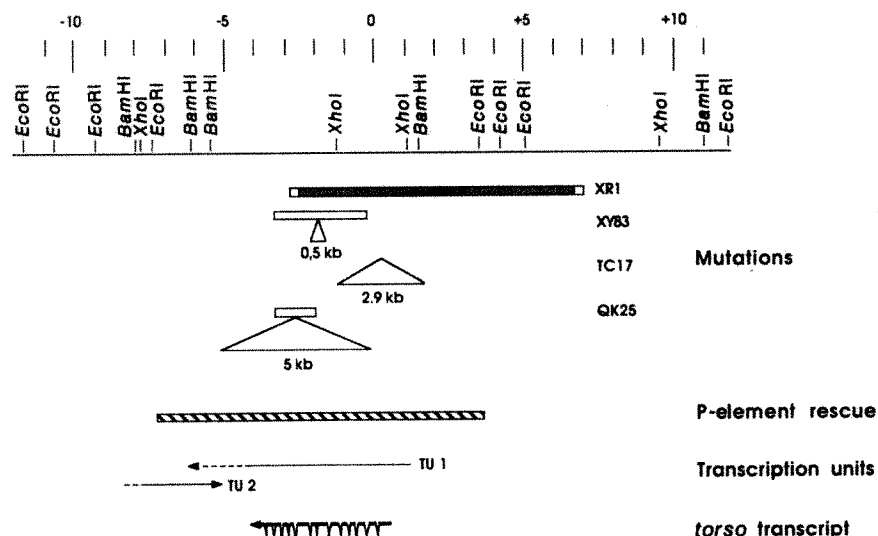
Here, we report that the product of the *torso* gene has structural similarities to receptor tyrosine kinases. This suggests a mechanism of the restricted *torso* action and provides a molecular explanation for the gain-of-function phenotype.

## Molecular cloning

The *torso* locus maps to the right arm of the second chromosome at cytogenetic position 43D3-E7. DNA from the *torso* locus was isolated by the P-element transposon tagging method<sup>8</sup> from *tor*<sup>TC17</sup>, a P allele obtained from T. Schüpbach. A genomic library was constructed from a subline of *tor*<sup>TC17</sup> in which many of the original P elements (over 50) had been removed by recombination. Two overlapping  $\lambda$  clones were isolated, which both contained 2.9 kilobases (kb) of P-element sequences and hybridized at cytological positions 43 E5-11 to wild-type salivary



FIG. 1 Structure of the genomic *torso* region. Numbers above the restriction map refer to distances in kb, the 0-point is defined by the P-insertion site. Mutations were mapped using Southern blot hybridization ( $tor^{XR1}$ ,  $tor^{XY83}$ ,  $tor^{QK25}$ ) and DNA sequencing ( $tor^{TC17}$ ).  $tor^{TC17}$  was isolated in a hybrid dysgenic cross (T. Schüpbach, personal communication),  $tor^{QK25}$  is an EMS-induced allele isolated by Schüpbach and Wieschaus<sup>2</sup>,  $tor^{XR1}$  is an X-ray-induced revertant of  $tor^{RL3}$  (ref. 7) and  $tor^{XY83}$  is an X-ray-induced revertant of  $tor^{Y9}$  (ref. 7). Embryos derived from mothers homozygous for these alleles develop a strong *torso* phenotype but show no other detectable phenotype. The filled bar represents the deleted region in  $tor^{XR1}$ , open bars above the triangles represent wild-type restriction fragments in which mutation-induced size changes were detected by Southern hybridizations. The 12-kb *EcoRI* fragment sufficient for P-element-mediated rescue is shown as a hatched bar. The two transcription units and their direction of transcription are presented as arrows. The exon-intron structure of the *torso* transcript, as inferred from the cDNA clone pCD7, is shown.



gland chromosomes. Fragments of these clones lacking P-element sequences were used to isolate  $\lambda$  clones from an Oregon P2 library, covering ~12 kb of DNA on either side of the P-insertion site (Fig. 1).

When the DNA structure of 35 ethyl methane sulphonate-induced and X-ray-induced alleles was analysed, three showed altered restriction maps in the cloned region as detected by Southern-blot hybridizations. The alleles  $tor^{QK25}$  and  $tor^{XY83}$  contain insertions of 5 kb and 0.5 kb, whereas the allele  $tor^{XR1}$  has a 9.5-kb deletion (Fig. 1) suggesting that this region contains sequences from the *torso* locus.

To confirm that we had cloned all of the sequences necessary for *torso* function, we used P-element-mediated germ-line transformation to rescue *torso* mutants. A 12-kb *EcoRI* fragment was cloned into the  $P^{PA}$  vector<sup>9</sup>, which carries the gene encoding alcohol dehydrogenase (*Adh*) as a marker and was injected into embryos from a strain mutant for both,  $tor(tor^{WK})$ , ref. 2), and *Adh* (*Adh*<sup>n2</sup>). Eleven independent transformant lines were obtained, all of which rescued both the ethanol sensitivity and wild-type *torso* function.

### The *torso* transcript

This 12-kb *EcoRI* fragment was used to screen a *D. melanogaster* embryonic complementary DNA library<sup>10</sup>. Two classes of cDNAs were isolated corresponding to two transcription units which are transcribed in opposite directions (TU1 and TU2) (see Fig. 1). Northern analysis revealed that the mRNA corresponding to TU2 is constitutively expressed during development (data not shown). Because only a portion of this transcription unit is contained within the rescuing *EcoRI* fragment, it is unlikely to represent the *torso* transcript.

Overlapping cDNA clones were isolated from TU1. The longest of these, pCD7 (3,063 base pairs (bp)), was used to examine the temporal expression pattern of the corresponding RNA on northern blots (Fig. 2a). A transcript of ~3.6 kb can be detected in RNA from 0–4-h-old embryos. After 4 h of development this signal is greatly reduced, but a very low level of expression is detectable throughout development after longer exposures. Adult females, but not males, show a high level of this transcript, consistent with expression in the ovary. In addition, a minor 5.5-kb transcript is visible after longer exposures (Fig. 2b). Neither the 3.6 nor the 5.5-kb transcript can be detected in RNA from the deficiency  $tor^{XR1}$  (Fig. 2b), indicating that both transcripts are derived from the *torso* region. Further analysis with smaller probes revealed that the 5.5-kb transcript is a 3'-extended version of the 3.6-kb transcript, suggesting that this minor transcript is the result of inefficient 3' processing of

the original transcript. Because TU1 has a pattern of expression that is consistent with a maternal gene, and because it is the only transcription unit entirely contained within the rescuing 12-kb fragment, we conclude that TU1 is the *torso* transcription unit.

### Expression of *torso*

The distribution of the *torso* transcript in ovaries and early embryos was examined by *in situ* hybridization to tissue sections. The transcript can be detected at low levels in the earliest stages of oogenesis (germarium, data not shown). It accumulates in the nurse cells until oogenic stage 10, with relatively low levels of transcript detectable in the oocyte (see Fig. 3a). After stage 11, when the nurse cells transport their contents into the oocyte very rapidly, the *torso* mRNA becomes evenly distributed within the oocyte and the nurse cells (data not shown). Immediately after egg deposition during early cleavage stages (Fig. 3b), the transcript is uniformly distributed in the embryo. During

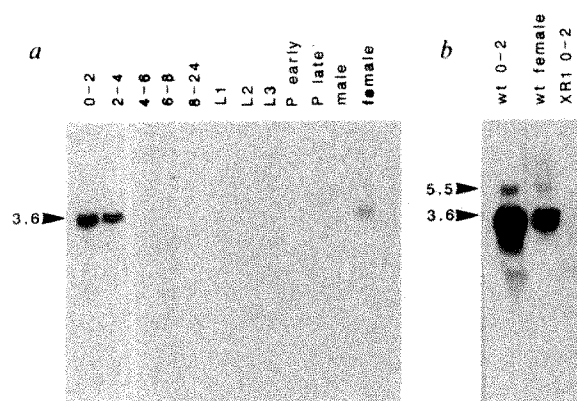


FIG. 2 Northern blot analysis. a, Development profile of the *torso* transcript. For the developmental stages indicated, 20  $\mu$ g total RNA were analysed by northern blot hybridizations. Abbreviations: L: larval stages, P: pupae. For size calibration, an RNA-Ladder (BRL, Bethesda) was used. b, Over-exposure of a northern blot containing wild-type and  $tor^{XR1}$  total RNA. Each lane contains 20  $\mu$ g total RNA.

METHODS. RNA was run on 1% agarose gels containing formaldehyde and transferred to Hybond nylon filters (Amersham, UK). Hybridization was performed according to the manufacturer's instructions, using nick-translated pCD7 DNA as a probe. RNA isolation and other standard techniques were as described<sup>34</sup>.



syncytial blastoderm (Fig. 3c) the RNA moves towards the periphery of the embryo, apparently accompanying segregation of cytoplasm from yolk material. At the end of syncytial blastoderm, most of the *torso* RNA is found in the cytoplasm underneath the nuclei. The intensity of the label decreases with increasing age of the embryo and after cellularization no signal above background can be detected. The distribution of the *torso* transcript resembles that seen for total poly(A)<sup>+</sup> RNA<sup>11</sup> and shows no apparent localization. The observed signal is specific, however, because embryos and oocytes from homozygous *tor*<sup>XR1</sup> flies lack any signal above background levels (Fig. 3b).

### The *torso* gene and protein structure

We have sequenced both the 3.1-kb cDNA and approximately 4.7 kb of the genomic *torso* region (Fig. 4). The cDNA is truncated at both the 3' and 5' ends, but appears to contain the entire coding sequence (see below). The sequence ATCATTC, which fits the heptanucleotide consensus sequences ATCA(G/T)T(C/T) found at the 5' end of many *Drosophila* mRNAs (ref. 12), lies 105 bases 5' to the beginning of the cDNA sequence, suggesting that this may be the 5' end of the mRNA. In addition, a consensus TATA and CAAT-box (ref. 13) are 26 and 79-bp upstream from the presumed transcription start site. A consensus polyadenylation signal AATAAA, which is normally present some 10–30 residues upstream of the poly-A tail<sup>14</sup> directly follows the 3' end of the cDNA. The deduced length of the *torso* mRNA is therefore 3.2 kb, which is in good agreement with the 3.6 kb determined from gel migration and assuming a poly(A) tail of some 100 bases. Comparison of the cDNA and genomic sequences indicates that the *torso* gene contains at least 13 introns, which are, from 5' to 3', 67, 152, 60, 56, 68, 56, 57, 61, 67, 54, 60, 56 and 58 bp long.

Conceptual translation of the complete cDNA sequence in all three reading frames reveals a single long open reading frame of 2,936 bp that is preceded by an in-frame stop codon and

terminated with two stop codons. Within the first 82 codons after the stop codon which defines the start of the open reading frame there are three ATGs. Only the first exhibits a match to the consensus translation initiation site described for *Drosophila*<sup>15</sup>. Starting with the first Met, the open reading frame consists of 2,768 base pairs, encoding a 923 amino-acid (aa) protein with a predicted relative molecular mass of 105,162.

A hydropathy profile<sup>16</sup> of the predicted polypeptide (Fig. 5) displays a strongly hydrophobic region of 22 amino acids between residues 400 and 422. By the criteria of Klein *et al.*<sup>17</sup>, this hydrophobic segment has a high probability of spanning a membrane. This segment is preceded by a positively charged lysine residue that could interact with the polar groups of membrane lipids and is followed by two charged arginines that could serve as a transfer-stop signal. There are 15 potential glycosylation sites found in the sequence, twelve of which are amino-terminal to the hydrophobic segment. A shorter hydrophobic region of 17 amino acids is present at the amino terminus of the protein and exhibits several features typical for leader sequences which are important for transport into the endoplasmic reticulum (ref. 18). A possible cleavage site for the signal peptidase, deduced from the -3/-1 rule<sup>19</sup>, is between residues 20 and 21. These features of the primary structure suggest that the *torso* protein is a transmembrane protein consisting of an extracytoplasmic domain of 380 amino acids, a transmembrane domain of 22 amino acids and a cytoplasmic domain of 402 amino acids.

### A putative receptor tyrosine kinase

Comparison of the *torso* polypeptide with published sequences indicates significant homology between *torso* and proteins with tyrosine kinase domains. Alignment of the *torso* protein sequence with other protein tyrosine kinases reveals a high degree of amino-acid identity over the entire catalytic domain (Fig. 6), including the invariant lysine which is believed to be

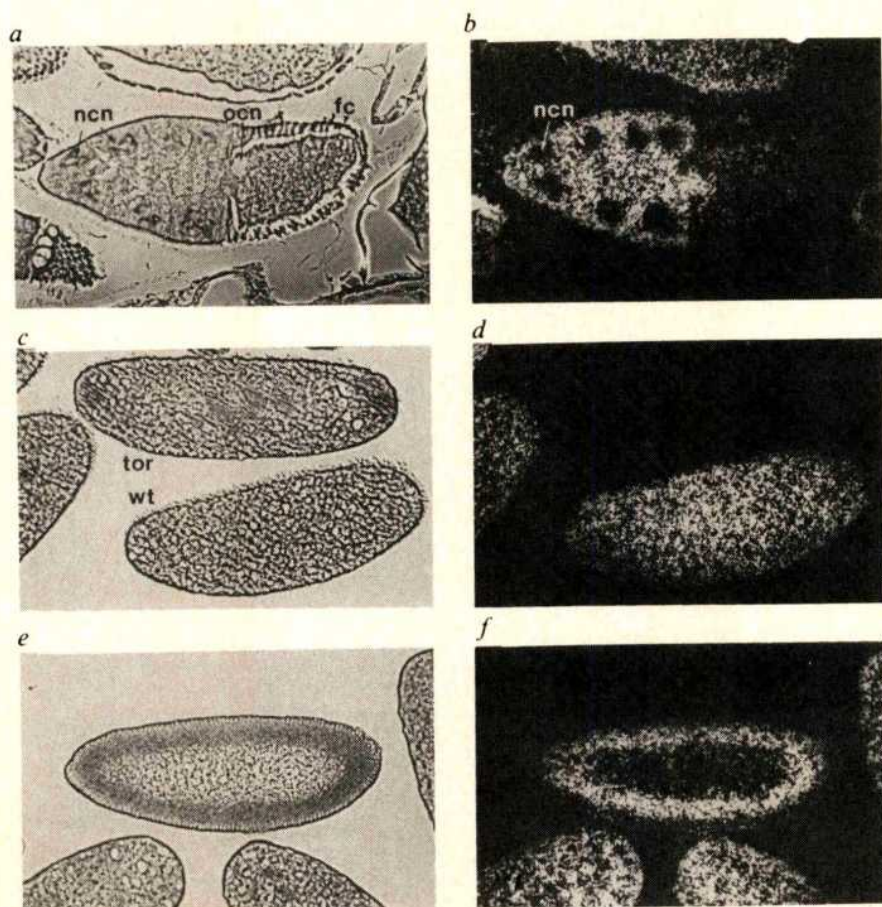


FIG. 3 Spatial pattern of the *torso* transcript during oogenesis and early embryogenesis. *a* and *b*, Late stage-10 follicle. The nurse cell complex is to the left, the oocyte in the right half of the egg chamber. The dorsal side is up. No labelling can be detected in the follicle cells and the oocyte nucleus. *c* and *d*, Early cleavage (<2 h) wild-type (wt) and *tor*<sup>XR1</sup> (*tor*) embryo. *e* and *f*, Syncytial blastoderm embryo (<4 h). Most of the label is in the cytoplasm below the nuclei. No label was detected at any oogenic or embryogenic stage in sections from homozygous *tor*<sup>XR1</sup> females (only shown for early cleavage stage). Abbreviations: ocn: oocyte nucleus, nc: nurse cell nuclei, fc: follicle cell.

METHODS. Preparations of tissue sections and hybridization conditions using <sup>35</sup>S-labelled pCD1 derived antisense RNA were as described<sup>35</sup>. Embryos and ovaries were treated identically except devitellogenesis was omitted for ovaries. Oogenesis stages are those described by King<sup>36</sup>.



1 tccatgttgagtggttccgactactcgaatcgatgcaacaattgagtcaccaatgtaatcgagcgttctatcagaataacctgaatcagaggcaactgag  
101 gtcattgtgcatcttgcgaatgcgtataaaggaataacctgtattgtcagctctgttccaattgaaatggaatcagggaaagtgtgacacgcaaaagtaca  
201 atgtggcgcaaatatgcatagttgttttttgggttaatttcgaatatttgccgccaacatggttctcagggatggaagcgaccagcagcgttctgattt  
301 atcgggttgcgtcaatacaagctcttttggcgcaacagatcatagaagattgaaatacaaacgttgggtggtatcacaataaagaaatgcgaagagtt  
401 atcgaagtcaatgttctatgcttcccaagccctggcgctactatgacggagtagcgatcccaatttttgcatttcaaatgaccctagtccaatccc  
501 atccatcagatcaagtaacatcagatgagtgagtggaacaaatacaaacacaaactagcgcaaaatatttcaacgaatccgcccgttctcatca  
601 ttgctccttctcgtcgtcttttaccatcgatttttggcctcagtttctcagttcttctcagttcagcaagataatccggaagagcggcggtattg  
701 ATCTCGAAAGTGTGAATCTAGAACTAAGTATTTCGAATTTGGTGTGTGTTATTGAGTGTGTATCAAGTGGCGTGGCGAAAGTGCAATAAATCTT  
801 AAGTCGGTTTTAAACAAATACATTTTCATCCAACACACACACCGCACACACGAAGAACTAAAGAAGGGTTTCAAAAGCAGCAACGAAATGCG  
1  
901 TTTATTTCTATGCGAAGTACGCATTTATCTTCTGGTTTTCGTGGGAAGCAATCAAGGTGAAATGTTGCTAATGGCAAAATCTCTCAGGATAAGACGCT  
2 L I F Y A K Y A F I F W F F V G S N O G E M L L M D K I S H D K T L  
1001 TCTCAACGTCACCGCTTGCACCCAGAAATGTCTGGAAAAGGGCAGATGgtatgtttacacccttagaatgttggaaactccataacccaaagtggatg  
36 L V T A C T Q N C L E K G O M  
1101 cttctttctcagacagGATTTCGAAGCTGTTTAAAGGACTGCAGGATTAATGGAACATTTCCCGGGCTCTCCGCAAGTGCAGGAAAAGTACCAGATG  
52 D F R S C L K D C R I M G T F P P G A L R K V Q E N Y Q M  
1201 AACATGATCTGCCGACGAGTCGGAATCGTTTTCGAATAGTATGGGTGCAGCAGCGGGAACCGAGCGGCTCCAAATGCCACTACATAATCC  
80 N M I C R T E S I V P I D M V Q H S R G T E P A I  
1301 GGTGGATGCTGTCAGGACGACAAAGAAACCGCTTTACCTGgtcagatattagatgtaaacctattactatttcttaaaataccattttatt  
113 R V D A V K D D T L Y L  
1401 tgatgaatttctagaaaaagatgtactatgtagtcacgaacctaatgtttcgttttacaagtggtatcgctcttttctcagacttattcgtctgcagT  
  
1501 CTGATGACAACCTTTCTCATCTGCCGGGATGGAGTCCAACCTACCCACAACATCACCGCCCTGGCGATGCACGGAGATGGCAGCTACTCTTGATAGC  
129 S D D I L P L G L E S M S T H M I T A L A M H G D G S Y S L I A  
1601 AAAGGACCAAGCTTCCGACCTCATCCGAGGCTATCAGCCCAAGGAGCGGTGAATCTGCTGGGTTTGTCCCCAACAGCAGCAGCTGCAT  
163 K I D T F A T L I R G V Q P S K M G A V N L L R F V P Q P D L H  
1170 CACATTGCTGCCAAATCGAGTGAAGCCATCGCGGgttagtgcctcagtttaatacaatcaacgatctacgaacctcttctcatattccagAGA  
136 H I A A E I E W K P S A  
1801 GCAATGCTATTTCGACATGGTGTCTGATTAACCAACACGCTGAATATGGACGAGCCATGGAGGTGCAGTTCGGGATgtgagtagtgcagacgccc  
209 S N C Y F D M V S Y S T N S V N M D E P L E V Q F R D  
1901 ctgcaatgtagagcagcatttcttactgcagCGCAAAAGCTGTACAGGCACAGCGTGGCAACATTTGGAATTTGACAAACAGTATCACGTTGGCG  
236 R K K L Y R H T V D N L E F D K Q Y H V G  
2001 TAAGAAGCGTGAACATAATGAATCGACTGGAGAGCGATCTGCACTGGCTGCAATCGCTTTCGAAGCTGCTGGATTGGTATCCCTATACACACT  
257 V R T V N I M N R L E S D L Q W L P I A V P S C L D W Y P Y M Y T L  
2101 CTGCGTgaggttctcgaagtcctataataataataataataataataatgttccacccttctcagCACCCTAAGCCAGAGAACTTACTG  
291 C P P H K P E M L T  
2201 TGACCCAGACGATCTGCGCAATATTTGGCCCTGAACATCACCTGGCGGCTCCAGATACCTGGCGGATAACTATACACTTCACATCTTGATCT  
301 V T I L P N I L M I T W A R P R Y L P D M Y T L H I F D L  
2301 ATTCAAAGGAGTACGGAGCTAACTATACACTTGACCAAAACAGGAGCCACTTCTATGTACCAAGATCCGGTACTGGTTCCTTTCGAAGTACAT  
335 F K G G T E L M Y T L D Q M R S H F Y V P K I T V L G S H F E V H  
2401 TTGGTGGCCAGTCCGCGAGCGGAAAAACGATCCGCTTGACGTTGGACAAGGTCTCTCAGGTTGTTGCTGAGCGTgagtagcgtgaggaat  
368 L V A Q K M V S G L T L D K V P R G V L L S  
2501 aaatatattagacatccatttccatccattgaacagAGGCAACATGGTCAAGTTGGTACTCTTTATTATCGTCCCAATATGCTGCATTTGATGCTGTGC  
394 E G N M V K L V L F I I V P L C  
2601 TCCTGACGTTCTGCAAGGAAATCGTTCCGAGGTTGAGCGCTGCAATGGAGCGTAAAGGCGGAGGCGAGTGAATTTATCTCTCTCCCTGATGACA  
416 S L T F C R R N S E V Q A L Q M D A K D A K A S E F H L S L M D  
2701 CGAGTGGCTGCTGGTCAACCTCTGCGCAACGAGATCTGGAATTAATGGACGAGCTGGAGGTGGAGCCACCTCGGTGCTCTCAGGATGCTCTCGG  
449 S S G L L V T L S A N E S L E V M D E L E V E P H S V L Q D L L G  
2801 CGAAGGAGCCTTTGGCTTGGTGGCAGCTGGAGTTTACAAGAAACGCCAAGTGGCCGTCAAGTTGCTGAAAGgttgtaccatttggtaggaagtccttga  
483 E G A F G L V R R G V Y K K R Q V A V K L L R  
2901 ataattcaggaatttttctcctcagagATGAACCAACGAGGAGCGTATATCGCTTCAAGTGCAGAAATTCAGATGCTCAAGCCGCTGGGCAAGCATC  
506 D E P N D E D V Y A F K C E I Q M L K A V G K H  
3001 CAAATATTGGGTATCTGGGATCTGGGATCTCACTCGTTTACCAACAGGATGATGTTGCTAATGAATCTGACGCTTGAAGCCCTGCAAGCTTTTACG  
530 P N I V G I V G Y S T R F S N Q M M L L I E Y C S L G S L Q N F L R  
3101 gtaagtattttttatcatatttggaaacaaagtgtatagtaaaaaatgattacttagTGAGGAGTGGAGTTTCAAGCAGGAGCAAAATGCAATTGG  
564 E E W K F R Q E Q N A I G  
3201 ACTTAAGAAGACCTTGAACAGAGCTGGACAAACGCGGTTTAAACCGATCCCTAGAAATTCATCATGATGCGATAGAGGATCAACAACTCGATG  
577 L K K N L E A G Q N V D N R R F N R L P R N S I H D R I E D I N N S M  
3301 CTGTCCACTGTGAAGAGGAGTGAATCGGATCAGACACACTCAAGTCGATGTGAGACCTACACCTTCACTCGAATAACCAATGCAACCAAGG  
610 L S T V E E S D Q T H S R C E T Y T L T R I T N A A D N K  
3401 GCTATGGCTGGAGGACATGAACACATCGGTGGGAGTTACTTCCCAACACCGCTGAAGCTCCAAAGGATCAGCCAAACGGAAGCTGAAGCCGAGCC  
643 G Y G L E D I E N I G S Y I P K T A E A P K D Q P K R K L G Q P P  
3501 CAAGAAGACTCTGAAGCAGGATTTCAATCGGACAAAGAGCGAATCTTTGAGAACAAGGAATCTTTGATTGCTTGACTCATCGGATACCAAGCC  
676 K K D S K D F K S D N K K R I F E N K E Y F D C L D S D T K P  
3601 CGAATACCACTGAAATATGCAATTTGCTAGACATCGCCCAACAGGTGGCGGTGGGAATGtagcctacatctaactctacccataatgtaattaaaaag  
710 R I P L K Y A D L L D I A Q V A V G M  
3701 tgtaacatgtatcatttgcatttttagGAATTTCTGGCCCAAAAGTAGTGATAGGAGTCTGGTGGCCGGAATGTTAATCTCCGTAGATCGCA  
730 E F L A Q N K V V H R D L A A R N V R L S R D V Y H E  
3801 GCATCAAGATAGCAGATTTTGGgtgggtatctctgaaagtccaatgattagttatattgtgttttttttttaagGCTGAGTCGAGATGTGTATCATGA  
754 S I K I A D F G L S R D V Y H E  
3901 GAACGTGTACCGAAGTCCGAGGAAGTGGCAAGCTGCCATCAAGTGGCTGCGCTGGAGTCCCTACCCACCAGGTGTACACAGCTCAGAGCGATGg  
770 N V Y R K S G G S G K L P I K W L A L E S L T H Q V Y T S Q S D V  
4001 taagtataatttttgaagtccgaagcgatttttgggtgatatcttaccctcttagTTGCTCTTTGGTGTGCTGCTTGTAGATCAGCCTCTCG  
803 W S F G V L L Y E I T T L  
4101 GTGGAATGCCATATCCGTCGGTGTCTCCAGTGTCTTTCAGCTACTGCGACAAGGTATCGGATGAAGCGAGGAGGTGTACGCAAGAAATgtg  
816 G G M P Y P S V S P S D L L Q L L R Q G H R M K R P E G C T Q E M  
4201 ggtatcagtgcttagcgaagtacataattgttgatcacatttgccttaactcagGTTTTCCCTGATGGAAGCTGCTGGAGCTCGGTGCTCAGCAGCGG  
849 F S L M E S C W S S V P S H R  
4301 CAACATTTCCGCCCTTAAACACAGACTTGGTGGCATGATTTTGGCCATAACGATGTTCCAGAAAGCTGAACAACTGCAAGCTGCAACCGAGTCAAA  
864 P T F S A L K H R L G G M I L A T N D V P E R L K Q L Q A A T E S K  
4401 ATTAAGTCTGTGACGGTCTAAACAGgtgaagtaatttttaaaagacaattgtatagcttactttctaccttttaacatttagTAAAGTGGAGCAAGT  
869 L K S C D G L N S K V E Q V  
4501 GCCATGCGAGGAGAGCTATACCTAGAACCTTTGAATTAATAGTCTATTGCTTCAAGATTATAATGAACAGGTGCAATACATTCTAAATTCGAGTTCAAT  
912 P C E E E L Y L E P L N - -  
4601 TCAGACAAATGCTATGCCAGATAACCTTGCATAGCAataaacattaaagctagcctaagaaaaataaattttctttttataagaaaaaccaagatat  
4701 ttatagagtaaaactctagcagtgcccttatgtctcatagaaa

FIG. 4 Genomic and cDNA nucleotide sequence of the *torso* gene and the predicted *torso* protein sequence. The nucleotide sequence of the cDNA clone pCD7 is in upper case. Introns and genomic sequences not represented in the cDNA clone are in lower case. The predicted amino-acid sequence is shown below the nucleotide sequence, commencing at nucleotide (nt) 897. Amino-acid residues are numbered in italics. A putative CAAT (nt 518) and TATA box (nt 570), the conserved heptanucleotide at the presumptive transcription start site corresponding to the consensus ATCA(G/T)T(C/T) (ref. 12) (nt 597), the in-frame stop codons (nt 726, nt 4,537, nt 4,540) and the AATAAA polyadenylation signal (nt 4,636) are underlined. The putative transmembrane sequence is doubly underlined. Possible N-glycosylation sites<sup>37</sup> (consensus: N-X-(Ser/Thr)-X, with N as the glycosylation site and X any amino acid except Pro) in the amino-terminal half of the protein sequence are in bold face. The P-insertion site of the *tor*<sup>TC17</sup> mutation is between nt 944 and 945.

METHODS. Sequences were determined by dideoxy sequencing<sup>38</sup> using Sequenase (United States Biochemical Corporation) according to the manufacturer's instructions. Two strategies were used to prepare single-stranded DNA templates for sequencing. A genomic *Xba*I (nt 1411)–*Sac*I (nt 4283) fragment was randomly sheared by sonication and cloned into M13mp10. The rest of the genomic fragment and the cDNA was sequenced by cloning defined restriction fragments into M13mp18 or M13mp19 vectors. The complete sequence of both strands was determined for the genomic sequence. Three bases differ between the genomic sequence and the cDNA clone, probably as a result of reverse transcriptase errors during the construction of the cDNA library. In the cDNA, nt 707 is G, nt 2,320 is T and 2,568 is G. The resulting amino-acid change is Ile (405) to Val. We used the amino-acid sequence derived from the genomic sequence for further analysis. Sequence analysis was carried out using PCGENE software package (GENOFIT, Switzerland).



involved in the phosphotransfer reaction to tyrosine<sup>20</sup>. The homologies between the *torso* tyrosine kinase domain and the catalytic domain of other tyrosine kinases range from 42.4% for *ret* (ref. 21) to 29.9% for *eph* (ref. 22). This overall conservation between the *torso* peptide and tyrosine kinases derived from evolutionarily distant organisms such as mammals, strongly suggests that the *torso* protein functions as a protein kinase.

In addition to amino-acid identity within the tyrosine kinase domain, the *torso* sequence shares structural features with the subclass of receptor tyrosine kinases (RTKs). These molecules contain a relatively large extracellular domain, a single transmembrane domain, a juxtamembrane domain of 41–50 amino acids adjacent to the tyrosine kinase domain and a carboxy-terminal tail of variable length (reviewed in ref. 23). Because all these features are also present in the *torso* protein, it is tempting to speculate that *torso* encodes a receptor tyrosine kinase. As in three other RTKs, the platelet-derived growth-factor receptor (PDGF-R), macrophage growth factor receptor (CSF-1-R), and the putative receptor *c-kit*, which have been classified as type-III RTKs (ref. 23), the catalytic domain of the *torso* protein is interrupted by a stretch of hydrophilic amino acids. The class III RTKs are further characterized by an immunoglobulin-like structure in the extracellular domain which includes cysteines and conserved surrounding residues<sup>23</sup>. This immunoglobulin-like structure is absent in *torso* and comparison of the extracellular domains does not reveal similarities to other RTKs. Indeed, the amino-terminal half of the *torso* polypeptide shows no significant homology with any published sequence of the SWISS-PROT 6.0 and NBRF Protein 16.0 data-bases: *torso* therefore encodes a new type of receptor tyrosine kinase.

## Conclusions

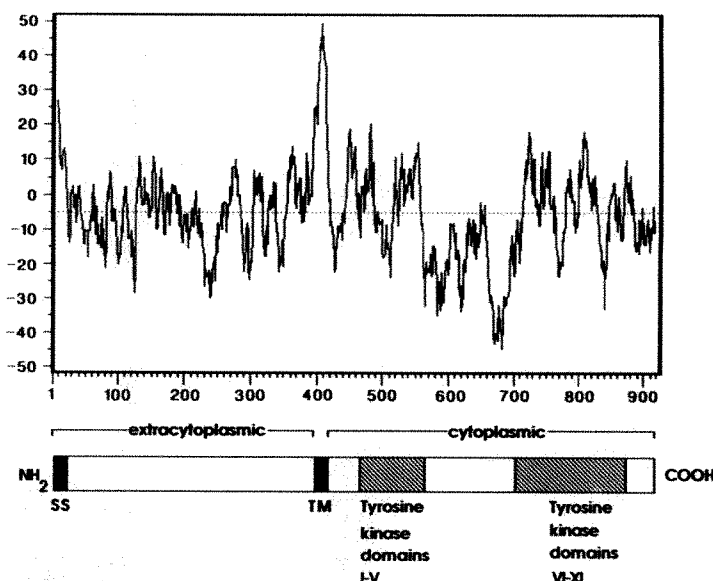
The proposed structure of the *torso* protein and its similarity with growth factor RTKs suggest that the *torso* peptide is a transmembrane protein with an extracytoplasmic domain acting as a receptor and a cytoplasmic domain containing tyrosine kinase activity. RTKs are normally activated by the binding of a ligand to the extracytoplasmic receptor, resulting in the phosphorylation of specific proteins and eventually producing a pleiotropic response, including proliferation and differentiation<sup>24</sup>. We propose that *torso* participates in such a signal transduction mechanism, and that the generation of the signal and its subsequent transmission from the membrane-associated *torso* product to zygotic target genes are functions of the other terminal-class genes. Another example of a signal-transduction

process involved in pattern formation may occur in the developing eye of *Drosophila*; the product of the *sevenless* gene, which is required for the determination of the R7 photoreceptor cell has also been identified as a putative RTK (reviewed in ref. 25).

Although genetic evidence indicates that *torso* is normally activated only at the poles<sup>7</sup>, cytoplasmic transplantation studies<sup>7</sup> and the homogeneous distribution of the *torso* mRNA suggest that the *torso* product itself is not localized. Instead, the local activation of *torso* may be achieved through a spatially restricted ligand molecule. By analogy with other RTKs which reside in the cell membrane, the *torso* product is probably incorporated into the cytoplasmic membrane of the oocyte and early embryo. Its extracellular domain would then be in the perivitelline space, the region between the oocyte cytoplasmic membrane (oolemma) and the vitelline membrane surrounding the oocyte. If so, the *torso* protein could serve as a receptor for a ligand molecule which is present in a spatially restricted fashion in the perivitelline space. Such a ligand molecule could reach the perivitelline space by secretion either from the oocyte or from the somatic follicle cells which surround the oocyte during oogenesis. In this respect it is notable that there are specialized follicle cells at the anterior and posterior end of the follicle<sup>26</sup>, suggesting the possibility that a subpopulation of follicle cells may be responsible for producing the spatially restricted molecule necessary for the activation of *torso*. A potential candidate for encoding such a ligand is *torso-like*, which is distinct from the other *torso*-group genes in that it is dependent upon the somatic rather than the germ-line cells of the ovary (H.-G. Frohnhöfer, personal communication). A precedence for transfer of information from the somatic follicle cells to the oocyte is found in the establishment of the dorso-ventral axis in *Drosophila*. In this system, a mutation in the soma-dependent gene *torpedo* results in a ventralization of the embryo<sup>27</sup>.

The identification of *torso* as an RTK also suggests a molecular explanation for the *torso* gain-of-function phenotype. Because it is likely that the *torso* protein is present all along the anteroposterior axis of the embryo, mutations which cause the kinase activity to become independent of ligand binding could create an ectopically active *torso* product and thereby produce a gain-of-function phenotype. Consistent with this interpretation, mutations that result in constitutively active tyrosine kinases have been isolated in other RTKs<sup>23</sup>. The dorsal-group gene *Toll* also exhibits both gain- and loss-of-function alleles that produce opposing phenotype<sup>28</sup>, and like *torso*, *Toll* encodes a transmembrane protein<sup>29</sup> which is believed to be activated in a spatially restricted manner<sup>30</sup>. Although the biochemical activity of *Toll*

FIG. 5 Hydropathy analysis and structural organization of the predicted *torso* protein. *a*, Hydropathy analysis. The 923 amino-acid long *torso* protein sequence was scanned using the program of Kyte and Doolittle<sup>16</sup>. Hydrophobicity results in positive and hydrophilicity in negative values. *b*, Structural organization of *torso* protein. Landmarks in the sequence are indicated schematically: signal sequence (ss) and transmembrane domain (TM) are cross-hatched, the tyrosine kinase domains (according to Hanks<sup>39</sup>) are hatched.



subdomain		I	II	III	IV
tor	473	HSVLLQDVLCAPGCVRRGVYK-----ROVAVKLKDEPNDDVYVAKCIGQLKAVGKRPVIGVGYSTRFSN			
ret	455	KNLVLGKTLCEPTCKVVKATAPHLKGRAGYTTVAVMKKNASPSLRDILSEFNVLKOV-NRPVHKLYGACSQD-GPILL			
src	265	ESLRLEVKLCQCCPCFVWCTWNG-----TTRVAITLTPGTH--SPEAFLOAGVMKLL-RREKLYQYAVVSE--EPIYI			
DER	685	AELRKGGLVLCAPGCVRRGVYKVPVEGEN-VKIPVAIKELKSTGAESSEFLRAYIMASE-EPVILLKLLAVCMS--SQM			
PDGFR	565	DQLVLGRITLCSAPGCVVEATAHGLSHSQATMKVAVMLKSTARSSEKQALMSKILKINSHLCPHILVVNLLGACTKG-GPIYI			
subdomain		V			
tor	549	LITYSIGSLQNFLEKMKFRQEQNAICLKKNLEQNVDRFRNRLPRNSIHDRIEDINNSMLSTVEESESQDTHSSRCETTY			
ret	536	IVETAKYGSIRGLFLESRRKVGPGYLGGSGSRSSSLDHPDERA-----			
src	337	VETMSKGSLLDFLKGETGKY-----			
DER	764	ITQLMPLGCLLDYVRNNRDK-----			
PDGFR	646	ITETRYGVGLVDVTLHRNKHTFLQRHNNKCPSPAEIYSNALPVGFSLP-SHMLNLTGESDGGYNDMSKDESIDYVPHLDHMKGIK			
tor	632	LTRITNAADNKGYLEGDIENIGGSYIPKTAEPKDPKRLKPKPKKSKQDFKSDNKKRIFENKEYFDCLDSSDTKPRIPLK			
ret	579	-----			LT
src	358	-----			LR
DER	784	-----			IG
PDGFR	729	YADIESPSYMAPYDNYVPSAPERTYRATLINDSPV-----			LS
subdomain		VI	VII	VIII	
tor	715	YADLLDIAQVAVGMEFLAQNKVVRDLAARNVLSVDRSIKTDVCLSRDVTYHENVYKSGCSGKPIKWLALSLTHOYTF			
ret	581	MGDLISFAMQISQCHVYLAEMKLVVRDLAARNVLSVDRSIKTDVCLSRDVTYHENVYKSGCSGKPIKWLALSLTHOYTF			
src	360	LPQLVDMAQIASGAYVERMNVVRDLAARNVLSVDRSIKTDVCLSRDVTYHENVYKSGCSGKPIKWLALSLTHOYTF			
DER	786	SKALNNWSTQIAKCHSYLEEKRLVVRDLAARNVLSVDRSIKTDVCLSRDVTYHENVYKSGCSGKPIKWLALSLTHOYTF			
PDGFR	766	YTDLVGFSYQVANGDFLAKNCKVVRDLAARNVLSVDRSIKTDVCLSRDVTYHENVYKSGCSGKPIKWLALSLTHOYTF			
subdomain		IX	X	XI	
tor	798	SQSDVWSFGVLLYRITTLGCGPTYSVSPSLLLOLRCHRMKPECTQDMSLMSCHSVSPSHRPTFSALKHRLGGMILAT			
ret	663	TQSDVWSFGVLLYRITTLGCGPTYSVSPSLLLOLRCHRMKPECTQDMSLMSCHSVSPSHRPTFSALKHRLGGMILAT			
src	441	IKSDVWSFGVLLYRITTLGCGPTYSVSPSLLLOLRCHRMKPECTQDMSLMSCHSVSPSHRPTFSALKHRLGGMILAT			
DER	867	SKSDVWSFGVLLYRITTLGCGPTYSVSPSLLLOLRCHRMKPECTQDMSLMSCHSVSPSHRPTFSALKHRLGGMILAT			
PDGFR	848	TLSDVWSFGVLLYRITTLGCGPTYSVSPSLLLOLRCHRMKPECTQDMSLMSCHSVSPSHRPTFSALKHRLGGMILAT			

FIG. 6 Comparison of the putative tyrosine kinase domain of the *torso* gene product with the corresponding domains of the products of the *ret* (ref. 21), human *c-src* (ref. 40), *Drosophila* EGF-receptor homologue (DER, ref. 41) and platelet-derived growth-factor receptor (PDGF-R; ref. 42) genes. Residues identical to the *torso* protein are in bold face and underlined. Classification into subdomains is according to Hanks *et al.*<sup>39</sup>. The lysine residue involved in the phosphotransfer reaction is marked by an arrow. Two sequence patterns which are thought<sup>43</sup> to distinguish all protein kinases from all other known proteins are marked with asterisks. The consensus pattern that specifies amino-acid specificity of the kinase to tyrosine residues, DLAARN, (in the catalytic domain VI) (ref. 39) is marked by #.

is not yet known, its similarities to *torso* support the hypothesis<sup>29</sup> that it functions as a receptor molecule.

The information generated by binding of the putative ligand molecule must ultimately be interpreted by the terminal zygotic genes. One maternally expressed gene which might be involved in carrying the signal downstream of *torso* is *l(1)polehole*. This gene has both maternal and zygotic functions and germ-line mosaic analysis<sup>5</sup> has shown that an embryo devoid of maternal *l(1)ph* mRNA but fertilized with a wild-type sperm develops a *torso* phenotype. The product of *l(1)ph* is likely to be identical to that of *l(1)raf* (refs. 31, 32), a homologue of the vertebrate serine/threonine kinase *Raf-1* (ref. 33). In addition, genetic evidence suggests that *l(1)ph* acts downstream of *torso* (M. Klingler, personal communication). Thus, signal transduction in the determination of terminal structure might involve a kinase cascade mechanism.

In summary, we propose that the *torso* gene encodes a receptor tyrosine kinase which is incorporated into the oolemma, but is not localized to the terminal regions. Its spatially restricted activity may be due to a local activation of the tyrosine kinase at the anterior and posterior ends of the embryo by binding of a localized ligand molecule to the extracellular domain of the *torso* protein. (In *torso* gain-of-function mutations, however, *torso* activity would be independent of ligand binding and the tyrosine kinase active everywhere.) Activation of *torso* would result in enhanced tyrosine phosphorylation of target proteins and the original signal would then be amplified by a cascade mechanism, in which at least one other kinase is involved. The activation of *torso* would ultimately lead to the regionalized expression of zygotic target genes, the next hierarchic level in the development of terminal structures of the *Drosophila* embryo. □

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# Production of self-absorbed synchrotron spectra steeper than $\nu^{5/2}$

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**SELF-ABSORBED** synchrotron radiation produced by electrons with a power-law distribution of energies has a unique spectral shape: intensity  $I_\nu \propto \nu^{5/2}$  (where  $\nu$  is frequency), irrespective of the power law index of the electrons<sup>1</sup>. This well-known result has been applied recently to the 'far-infrared turnovers' observed in the spectra of many radio-quiet active galactic nuclei (AGNs)<sup>2-4</sup>. It has been asserted that the measurement of a spectral index greater than 5/2 at frequencies below the turnover is incompatible with the physics of self-absorbed synchrotron sources. Several observations suggesting larger indices (steeper slopes)<sup>5-7</sup> between the far-infrared and millimetre bands have been interpreted as evidence that emission by warm dust, not synchrotron radiation, dominates the far-infrared spectrum. Rees<sup>8</sup> has, however, pointed out that this assertion is not necessarily true. Here we show that plausible electron energy distributions can lead to self-absorbed synchrotron spectra which are steeper than  $\nu^{5/2}$  over 1-1.5 orders of magnitude in frequency. This indicates that none of the existing observations are in fact incompatible with self-absorbed synchrotron radiation as the source.

Consider an electron energy distribution consisting of the sum of two power laws,

$$N_\gamma d\gamma = C_1 \gamma^{-p_1} d\gamma + C_2 \gamma^{-p_2} d\gamma \quad (1)$$

where  $\gamma$  is the electron Lorentz factor and  $C_1$  and  $C_2$  are constants. Without loss of generality we shall assume in the following that  $p_1 > p_2$ . As  $\gamma > 1$ , this implies that the steeper power law can become of importance only if  $C_1 > C_2$ . The synchrotron intensity in the self-absorbed limit is given by the ratio of the emission coefficient to the absorption coefficient, which for  $p_1, p_2 > \frac{1}{3}$  reduces to

$$I_\nu = \frac{j_\nu}{\kappa_\nu} = \frac{C_1 j_1 \nu^{-(p_1-1)/2} + C_2 j_2 \nu^{-(p_2-1)/2}}{C_1 \kappa_1 \nu^{-(p_1+4)/2} + C_2 \kappa_2 \nu^{-(p_2+4)/2}} \quad (2)$$

where the analytic expressions for  $j$  and  $\kappa$  are well-known (see, for example, ref. 1). The coefficients  $j_1, \kappa_1$  and  $j_2, \kappa_2$  depend

on  $p_1$  and  $p_2$  respectively, and on the cyclotron frequency  $\nu_B$ . Equation (2) assumes the ultra-relativistic approximation for synchrotron emission. As the effects that we are considering are likely to be caused by relativistic electrons with  $\gamma \geq 10$  (see below), this approximation is justified. For simplicity, we also neglect effects of polarization and pitch-angle distribution. A proper treatment of these effects<sup>9</sup> yields results that differ from our estimate by less than a few per cent. Defining  $\eta \equiv C_2 j_2 / C_1 j_1$ ,  $\chi \equiv C_2 \kappa_2 / C_1 \kappa_1$  and  $q \equiv (p_1 - p_2)/2$ , we find that the slope of the spectrum is given by

$$\alpha \equiv \frac{d \log I_\nu}{d \log \nu} = \frac{5}{2} + \frac{q \nu^q (\eta - \chi)}{(1 + \eta \nu^q)(1 + \chi \nu^q)} \quad (3)$$

Evaluating  $\eta$  and  $\chi$  using the standard expressions for power-law synchrotron emission and absorption, it can easily be shown that  $\alpha > \frac{5}{2}$  for all  $\nu$ , although the deviation from  $\frac{5}{2}$  becomes arbitrarily small in the limits of both large and small  $\nu$ . Differentiating equation (3) with respect to  $\nu$  yields the value  $\nu_m$  at which the maximum slope  $\alpha_m$  occurs. The latter is given by

$$\alpha_m = \frac{5}{2} + \frac{q[(\eta/\chi)^{1/2} - (\chi/\eta)^{1/2}]}{[1 + (\eta/\chi)^{1/2}][1 + (\chi/\eta)^{1/2}]} \quad (4)$$

This maximum slope depends only on  $p_1$  and  $p_2$ . In Fig. 1 we show a contour plot of  $\alpha_m$  as a function of  $p_1$  and  $p_2$ , from which we see that a significant deviation from  $\frac{5}{2}$  occurs if  $p_1 - p_2 > 2$ . To illustrate the width of the interval in frequency that is affected by the steepening, Fig. 2 shows a contour plot of the spectral index  $\alpha$  in the  $(\nu, p_1)$  plane, for  $p_2 = 3$ . (The latter value is thought to apply in AGNs, in which an optically thin synchrotron spectrum proportional to  $\nu^{-1}$  is usually observed.) In Fig. 2 the frequency is normalized to  $\nu_m$ , so that the result is independent of the actual values of  $C_1$  and  $C_2$ . The spectral index is significantly steeper than  $\frac{5}{2}$  over 1-1.5 decades in frequency.

Our numerical experiments for arbitrary electron distributions indicate that the main feature of the electron distribution responsible for a spectrum steeper than  $\nu^{5/2}$  is

$$\frac{d^2 \log N_\gamma}{d \log^2 \gamma} > 0 \quad (5)$$

Although we cannot prove this condition rigorously, it can be understood by considering the case of two power laws. For a power-law electron distribution, a photon with a given frequency is on average absorbed by an electron with an energy lower than the average energy of an electron that emits at this frequency. (See, for example, ref. 1, equation [6.52].) This means that the

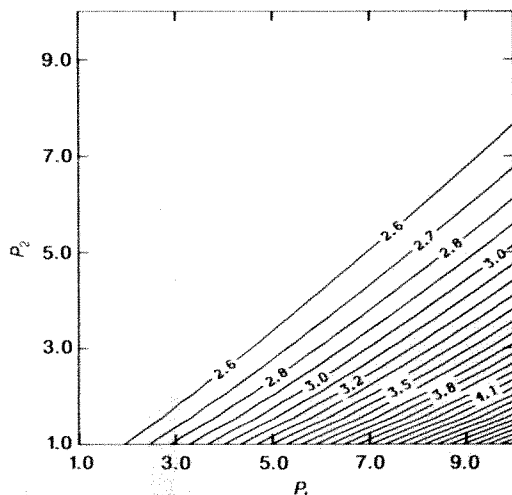


FIG. 1 Contour plot of the maximum spectral slope  $\alpha_m$  as a function of the power-law indices  $p_1$  and  $p_2$ .

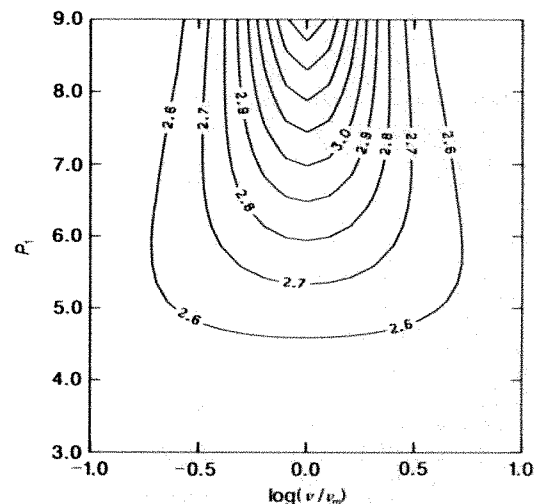


FIG. 2 Contour plot of the spectral slope as a function of frequency and  $p_1$ , for a fixed value of  $p_2 = 3$ . The frequency is normalized to  $\nu_m$ , at which the maximum  $\alpha_m$  occurs.



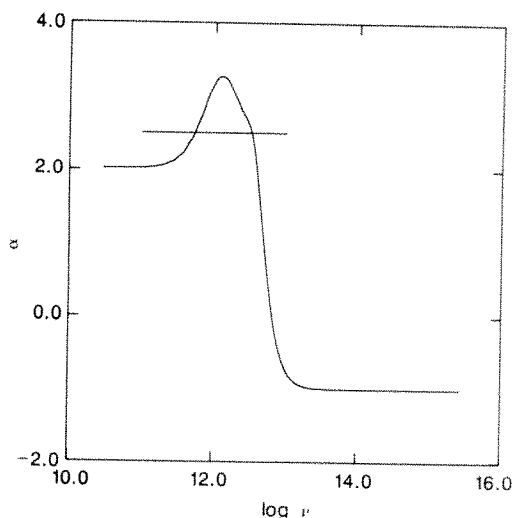


FIG. 3 The spectral slope as a function of frequency for a self-absorbed synchrotron source containing a distribution of relativistic electrons that is a combination of a power law and a relativistic maxwellian (equation (6)). At low frequencies the spectrum is thermal ( $\alpha = 2$ ); it steepens considerably before becoming optically thin ( $\alpha = -1$ ) at high frequencies. The illustrated spectrum is for  $\beta = 1$  and  $C_1/C_2 = 1$ . The horizontal line represents a spectral slope of  $\frac{5}{2}$ .

absorption coefficient in equation (2) (the denominator) switches to being dominated by the first term (power law 1) at a higher frequency than the emission term (the numerator), so that the spectral index tends to  $\frac{5}{2} + q$  rather than  $\frac{5}{2}$ . Thus a distribution in which the number of low-energy electrons is over-represented relative to a power law extrapolation of the high-energy distribution is likely to produce a spectrum steeper than  $\nu^{5/2}$  over some frequency range. Note that neither the distribution function nor the derivative of its slope need be monotonic.

How can a relativistic electron distribution with the properties necessary for  $\alpha$  to be greater than  $\frac{5}{2}$  be produced in an AGN? A significant steepening of the electron distribution at low energies, relative to a power law, occurs if (1) there is extra injection of relativistic electrons at low energies described by a steep power law, for example from Fermi acceleration produced by many weak shocks; (2) there is a tail of a relativistic maxwellian distribution connecting with the power law at low energies, a situation that arises naturally if the effect of synchrotron self-absorption on the relativistic electron distribution is taken into account<sup>10,11</sup>; or (3) the net cooling rate of the relativistic electrons varies with energy in such a way that condition (5) is fulfilled. This would require that the cooling rate decrease towards lower energies more steeply than can be described by a single power law, or that there is a cooling mechanism that acts only in a restricted energy range.

The third possibility seems rather unlikely, but the first two are physically quite reasonable. Case (2), the combination of a relativistic maxwellian and a power law, is difficult to solve analytically, but we show in Fig. 3 our numerical results for the spectral index  $\alpha$  as a function of frequency, for  $N_\gamma$  of the form

$$N_\gamma = C_1 \gamma^2 e^{-\beta\gamma} + C_2 \gamma^{-3} \quad (6)$$

where  $\beta$  is an arbitrary constant. The strong inflection point in the electron distribution at the transition between the exponential tail and the power law causes a sharp peak in  $\alpha$ , with a width of about a decade. For  $\beta \approx 1$  and a wide range in  $C_1/C_2$ , the inflection point in the distribution (6), at which the two terms become equal, occurs for  $10 < \gamma < 50$ . If the usual order-of-magnitude estimate of the magnetic field strength in an AGN of  $10^3$  gauss is correct, this range in  $\gamma$  corresponds precisely to the range in energy containing the electrons responsible for the synchrotron radiation just below the turnover<sup>10</sup>.

Before comparing with observations, we must mention that there are effects that could flatten the observed spectrum from the ideal self-absorbed synchrotron spectrum we have discussed above. Prominent among these are radiative-transfer effects (if the source does not have a very sharp edge) or inhomogeneity (if parameters vary over the source surface).

The lower limits on the spectral slope below the turnover in observed AGN spectra are derived<sup>6</sup> mostly from the 100- $\mu\text{m}$  and 1.3-mm fluxes. To compare this with our results we have calculated the maximum average  $\alpha$  obtainable over such a factor-of-13 range in frequency. We find that this maximum is rather independent of the details of the model, and is about 2.75–2.8. The high value of  $\alpha$  of 3.06 reported<sup>7</sup> for wavelengths between 155 and 438  $\mu\text{m}$  in NGC4151 can also be reconciled with our models because, as Figs 2 and 3 indicate, steeper slopes can be obtained over a smaller range in frequency. These results imply that there are at present no measured spectral slopes below the turnover for which synchrotron radiation cannot be the main emission mechanism.  $\square$

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## Anisotropic optical and X-ray emission in quasars

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IT is of fundamental importance in the study of quasars to understand whether or not their continuum emission is isotropic, because this affects the overall energy budget and can also help to distinguish between the different models proposed for their central regions<sup>1</sup>. The reported small range of emission-line equivalent widths in quasars have earlier been used to argue against a relativistically beamed or anisotropic continuum<sup>2,3</sup>. Here we use recent spectroscopic observations of 46 radio-loud quasars to show that the equivalent width of the [O III] 5,007-Å emission line is strongly anticorrelated with the degree of radio core prominence  $R$ , which has been found to be a statistical indicator of orientation relative to the line of sight<sup>4–6</sup>. This observed anticorrelation shows that the quasar continuum is anisotropic and provides important evidence in favour of relativistic-beaming unified schemes<sup>4,7,8</sup>. We also show that the strength of the [O III] emission line is independent of  $R$ , consistent with unified schemes.

In relativistic beaming models, the radio emission from the core can be strongly Doppler boosted at small angles to the line of sight, because the material is believed to be moving relativistically<sup>8</sup>. On the other hand, the extended radio emission is at most mildly relativistic. Thus in such models the ratio  $R$  of core emission to extended radio emission is a useful indicator of source orientation, with  $R$  increasing as the angle to the line of sight decreases<sup>4</sup>. Several statistical studies have shown the radio properties of quasars to be consistent with such a scheme<sup>4,5,9,10</sup>.

A principal argument against extending such a scheme to include relativistic beaming of the optical and X-ray continuum<sup>11</sup> derives from the small range of emission-line equivalent width in quasars<sup>2,3</sup>. As the line luminosity should not depend on orientation, relativistic beaming of the continuum should be reflected in a spread in equivalent width, with the high- $R$  objects having systematically lower equivalent widths than low- $R$  objects.

To investigate this problem using a homogeneous set of spectra, we observed 46 radio-loud, low-redshift quasars with the Faint Object Spectrograph<sup>12</sup> on the Isaac Newton Telescope. Because we wished to study relativistic beaming of both the optical and X-ray continuum, most of the objects were selected on the grounds of having been studied at X-ray energies (2 keV) by the Einstein Observatory. Nearly all such objects with  $z \leq 0.8$  were observed, and several other quasars were added to fill gaps in the observing schedule, usually on the grounds of ease of observation and membership of the 3C catalogue. We emphasize that no prior selection by emission line strength has been imposed other than that implied by the necessity that all objects have measured redshifts and are classified as quasars by Véron and Véron<sup>13</sup>.

The spectral resolution of these observations is about 18 Å and the wavelength coverage is 5,000–9,500 Å. In every object, lines of H $\beta$  and [O III] 5,007-Å emission are observable. We have measured [O III] in all but three objects; for these three we have upper limits to [O III]. Full details of the observations,

calibration and data reduction are given elsewhere<sup>14</sup>. We have taken X-ray and radio luminosities from previous compilations<sup>11,15,16</sup>, supplemented in a few cases with radio data from our own unpublished Very Large Array (VLA) observations.

Here we focus on the [O III] line fluxes and their correlations with  $R$ , the ratio at 5 GHz of the core luminosity to the extended radio luminosity  $L_{\text{ext}}$  in the quasar's rest frame. We use [O III] because it is easier to measure than H $\beta$  and is less affected by contamination with broad Fe II blends<sup>14</sup>; moreover, it is emitted further from the quasar nucleus (a few hundreds of pc rather than a few pc) and orientation-dependent obscuration effects should interfere less with its flux.

In Fig. 1a and b we plot [O III] optical and X-ray equivalent widths against  $R$  (X-ray equivalent width is defined as the ratio of [O III] line flux to the K-corrected<sup>11</sup> X-ray continuum at 2 keV). The spread in equivalent widths is at least an order of magnitude and, contrary to earlier suggestions<sup>17</sup>, there is a very significant anticorrelation between the equivalent width and  $R$ . The formal levels of significance are listed in Table 1. If we accept relativistic beaming models, this result provides strong evidence that some part of the quasar continuum emission increases when viewed at small angles to the line of sight. We have examined the possibility that the above anticorrelation could be a secondary effect of a primary correlation between equivalent width and extended luminosity together with a selection-induced anticorrelation between extended radio luminosity and  $R$ . Both a partial correlation coefficient analysis<sup>18</sup> (Table 1) and comparisons of pairs of quasars matched in extended radio luminosity show that the equivalent width:  $R$  anticorrelation is significant. Moreover, in a virtually complete subsample (13 objects out of 17) from quasars having a flux at 2.7 GHz  $\geq 1.5$  Jy (ref. 19) and a redshift  $z \leq 0.8$  in the RA (right ascension) ranges over which we have data, the rank correlation is  $-0.74$ , giving a significance level of 99%. This suggests that selection effects are not responsible for the anticorrelation.

We can use our data to investigate whether the line and continuum luminosities lend independent support to the relativistic beaming unified scheme. If the low- $R$  and high- $R$  objects are intrinsically similar but differ only in orientation, their [O III] luminosities should be similar for a given extended radio luminosity. We indeed find that the hypothesis that the two distributions are indistinguishable cannot be rejected by a Kolmogorov-Smirnov test at the 80% level for 13 pairs of core- and lobe-dominated quasars matched in extended radio luminosity. Earlier studies have shown that, again for a given extended radio luminosity, the core-dominated (high- $R$ ) quasars have more optical and X-ray continuum than the lobe-dominated quasars<sup>11,20</sup>. We find a similar result using our data. The

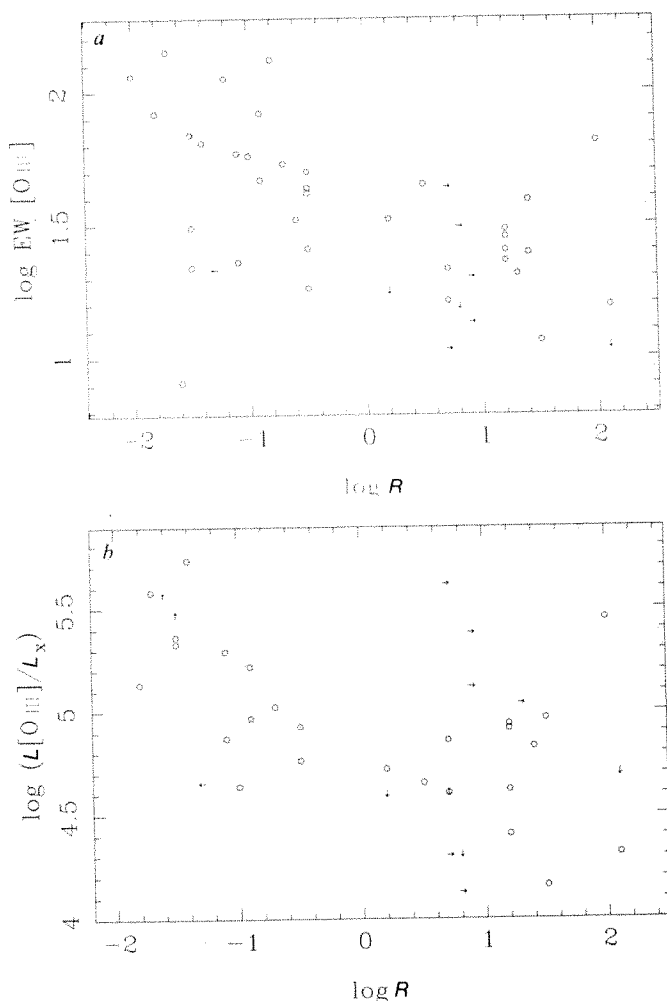


FIG. 1 a, Logarithm of optical equivalent width plotted against  $\log R$  (defined in the text). Measured points are shown as circles, limits as arrows. b, Logarithm of X-ray equivalent width ([O III] luminosity divided by 2-keV X-ray luminosity  $L_x$ ) plotted against  $\log R$ . Symbols are the same as in a.

TABLE 1 Correlations of various parameters with  $R$

Parameter	Rank correlation	Number of points	Significance (%)
EW [O III]*	-0.53	46	99.9
EW [O III]†	-0.52	36	99.9
X-ray EW [O III]*	-0.47	38	99.5
X-ray EW [O III]†	-0.55	27	99.5

Correlations using 46 points			
Parameters	Rank correlation	Partial RC	Significance (%)
EW [O III]: $R$	-0.53	-0.47	99.9
EW [O III]: $L_{\text{ext}}$	0.29	0.04	60
$L_{\text{ext}}: R$	-0.49	-0.41	99.5

EW: equivalent width.

\* Coefficients for all data, including upper and lower bounds.

† Coefficients excluding data for upper and lower bounds.

hypothesis that the distributions of continuum luminosity are identical can be rejected by the Kolmogorov-Smirnov test at the 95% level. Our conclusion is that, in addition to some isotropic emission, there is a component of quasar continuum that depends systematically on orientation.

There are at least two ways of producing directionally dependent continuum emission: by relativistic beaming of non-thermal radiation<sup>7,8</sup> or by thermal radiation from an optically thick disk whose axis is parallel to the radio axis<sup>1</sup>. These two effects dominate over different ranges of  $R$ : relativistic beaming dominates for  $R \geq 1$ , and disks for  $R \leq 1$ . Although we would argue that relativistic beaming is responsible for the extra brightness of high- $R$  objects, sometimes leading to blazar activity, whether or not the effects of directionally dependent disk radiation are noticeable at low  $R$  remains to be investigated.

Finally we consider whether objects that do not satisfy our quasar selection criteria should be added to Fig. 1. At one extreme there are BL Lac objects, whose lines are generally too weak to allow measurement of their redshift. These objects would appear in the lower right area of Fig. 1 because they are invariably core-dominated. We strongly suspect that at least some of these objects should be included with the quasars, and this inclusion would obviously reinforce the observed anticorrelation. At the other extreme lie radio galaxies, objects with very high equivalent widths and, usually, very low  $R$ . It has been argued<sup>21,22</sup> that these objects should also be considered as quasars, with their radio axes in the plane of the sky and their nuclear continuum sources and broad-line regions obscured. Although adding them to Fig. 1 would greatly increase the significance of the correlation, we feel that the case for inclusion of these objects has yet to be fully established.

We conclude that the similarity of [0111] strengths in core- and lobe-dominated quasars of the same extended radio luminosity supports models in which the only difference between the two types of quasar is viewing angle, together with the consequent Doppler beaming. The natural interpretation of our equivalent width:  $R$  anticorrelation is therefore that the optical continuum is anisotropic. This is an important result, for two reasons. First, emission models must take into account the fact that the different parts of the line-emitting region may see very different photo-ionizing continua. And second, the assumption that selection of quasars by optical brightness leads to samples with a random distribution of orientation angles<sup>23</sup> may not be valid.  $\square$

## Impact erosion of the primordial atmosphere of Mars

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**ABUNDANT** geomorphic evidence for fluvial processes on the surface of Mars suggests that during the era of heavy bombardment, Mars's atmospheric pressure was high enough for liquid water to flow on the surface. Many authors have proposed mechanisms by which Mars could have lost (or sequestered) an earlier, thicker atmosphere but none of these proposals has gained general acceptance. Here we examine the process of atmospheric erosion by impacts and show that it may account for an early episode of atmosphere loss from Mars. On the basis of this model, the primordial atmospheric pressure on Mars must have been in the vicinity of 1 bar, barring other sources or sinks of  $\text{CO}_2$ . Current impact fluxes are too small to erode significantly the present martian atmosphere.

Although a great deal of effort has been expended on the study of the craters produced by large impacts, little work has been done on the atmospheric effects of impacts. A beginning was made by Walker<sup>1</sup>, who showed that a projectile traversing the atmosphere of a planet could eject at most only a few times the mass of the atmosphere traversed. This conclusion generally agrees with that of Ahrens and O'Keefe<sup>2</sup>, who studied the effects of the airblast produced by the impact and of the early fast ejecta on atmospheric erosion. Previously<sup>3</sup> we used an analytical model to show that fast solid ejecta from the crater are similarly

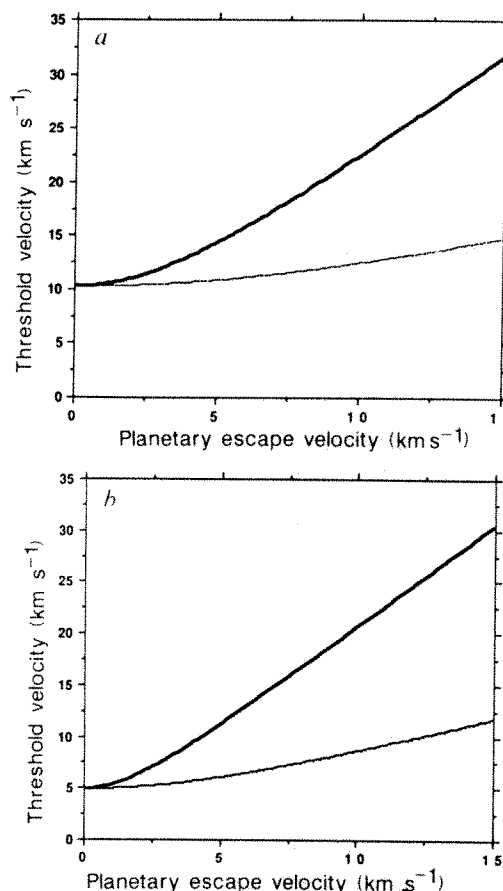


FIG. 1 Threshold impact velocity for some (light line) and most (heavy line; from equation (1) of the text) of the vapour produced in an impact to reach escape velocity of the planet on which it strikes: a, for silicate projectiles; b, for ice projectiles.

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unable to eject more than a few times the atmospheric mass traversed by the projectile. However, we show here that the vapour plume created by high-speed impacts is extremely effective in ejecting atmospheric gases—for sufficiently large impacts the vapour plume may eject the entire airmass above the plane tangent to the point of impact, a result in general conformity with those of refs 4 and 5. Atmospheric erosion by impacts may therefore play an important part in the evolution of atmospheric pressure, especially on Mars because of its low gravity and small radius.

Although much of our work is based on detailed computer modelling, it is easy to construct a simple analytical model of atmospheric erosion by impacts that seems to capture most of the essentials of the process. Only a few basic equations are needed if one is willing to overlook uncertainties and subtleties that could change the results by factors of about two. The first essential feature for atmospheric erosion to occur is that the projectile must strike the planet with enough velocity for a vapour plume to form. The plume must also expand at a speed faster than the planet's escape velocity,  $v_{\text{esc}}$ . A model of vapour-plume expansion<sup>6</sup> gives the mean (mass-averaged) velocity of expansion as  $[2(E - H_{\text{vap}})]^{1/2}$ , where  $E$  is the specific energy of the vaporized projectile and target material and  $H_{\text{vap}}$  is the vaporization energy, taken here to be 13 MJ kg<sup>-1</sup> for silicates and 3 MJ kg<sup>-1</sup> for ice. The maximum expansion velocity of the gas cloud is approximately a factor  $[2\gamma/(\gamma - 1)]^{1/2}$  ( $=2.82$  for  $\gamma = 4/3$ , where  $\gamma$  is the ratio of specific heats) times faster than this, so use of the mean velocity is a conservative assumption. The specific energy  $E$  is, from the Hugoniot equations, equal to  $u^2/2$ , where  $u$  is the particle velocity behind the shock. The maximum particle velocity  $u$  is  $v/2$ , where  $v$  is the impact velocity, by the planar impact approximation when both target and projectile are nearly the same material, so  $E \approx v^2/8$ . After a little algebra, the threshold impact velocity  $v_{\text{min}}$  for most of a vapour plume to exceed escape velocity can be shown to be

$$v_{\text{min}} = \sqrt{8 \left( \frac{v_{\text{esc}}^2}{2} + H_{\text{vap}} \right)} \quad (1)$$

Figure 1 illustrates this threshold for both silicate and icy projectiles, along with the more optimistic threshold at which some of the expanding vapour plume reaches escape velocity. We believe the more conservative limit in equation (1) is to be preferred for atmospheric-loss computations.

In the case of Mars,  $v_{\text{min}}$  is 14.3 km s<sup>-1</sup> for silicate impactors and 11.1 km s<sup>-1</sup> for icy impactors. These minima are less than the estimated r.m.s. impact velocities<sup>7</sup> with either Earth-crossing asteroids (19 km s<sup>-1</sup>), periodic comets (21 km s<sup>-1</sup>), or parabolic comets (42 km s<sup>-1</sup>). It is slightly larger than the mean impact velocity<sup>8</sup> with Mars-crossing asteroids (10 km s<sup>-1</sup>). Here we assume that the flux of impactors fast enough to produce a vapour plume that exceeds Mars's escape velocity is half of the total flux. During accretion, impact velocities were undoubtedly

lower so that at that time Mars could have accumulated its original atmosphere.

The second factor in estimating whether or not an impact can remove the atmosphere is that if the vapour plume is to carry away the atmosphere, the mass of vapour from projectile and surrounding vaporized target (if any) must exceed the airmass  $m_p \approx 2\pi P_0 HR/g$  above the plane tangent to the impact. This relation is supported by our more detailed numerical studies. Thus, we take

$$m_* \geq \frac{2\pi P_0}{g} HR \quad (2)$$

where  $m_*$  is the projectile mass just capable of removing the atmosphere above the tangent plane,  $P_0$  is the surface atmospheric pressure,  $g$  is the acceleration of gravity,  $R$  is the radius of the planet, and  $H$  is the atmospheric scale height. In equation (2) it is assumed that the projectile and an equal mass of the target are vaporized and shocked to the same total internal energy. Although other assumptions could be made, this is one of the most conservative because it neglects a possibly significant quantity of vaporized target that may expand fast enough to reach escape velocity itself.

A potentially important effect neglected in equation (2) is the concentration of atmospheric mass at low angles from the horizon. The vapour plume expands nearly hemispherically, and thus may couple inefficiently to the atmospheric mass which is concentrated toward the horizon. Note that after the vapour plume has expanded to a few tens of the projectile diameter, adiabatic cooling condenses much of the vaporized rock into a dense, fast-moving dust cloud that follows nearly ballistic trajectories and sweeps the ambient atmosphere before it. This effect is largest for small values of  $H/R$ . Our detailed numerical computations suggest that this effect is minimal for Mars, but could be important for the Earth or Venus.

For Mars, the smallest projectile that can remove the entire airmass above the tangent plane at the present time is  $\sim 4 \times 10^{13}$  kg, or a silicate object  $\sim 3$  km in diameter. Note that equation (2) does not take account of possible enhanced vaporization in oblique impacts — such an angle-dependent phenomenon might reduce  $m_*$  by a factor of perhaps five.

These results for the mass of the projectile necessary to remove the atmosphere above the tangent plane must be supplemented with the flux of projectiles to compute the evolution of atmospheric pressure with time. The present cumulative flux (number s<sup>-1</sup> m<sup>-2</sup>) of projectiles with mass greater than or equal to  $m$  is parameterized by  $N_{\text{cum}}(m) = am^{-b}$ , where  $a$  and  $b$  are constants. Unfortunately, neither the overall flux,  $a$ , nor the slope of the distribution,  $b$ , are well-known. Values of these constants that predict the correct present lunar crater distribution via the revised Schmidt-Holsapple scaling law<sup>9</sup> are  $a = 1.55 \times 10^{-23}$  kg<sup>b</sup> m<sup>-2</sup> s<sup>-1</sup>,  $b = 0.47$ . The slope of the crater distribution on the martian plains is the same as the lunar distribution<sup>10,11</sup>, and the overall cratering rate on Mars at present is

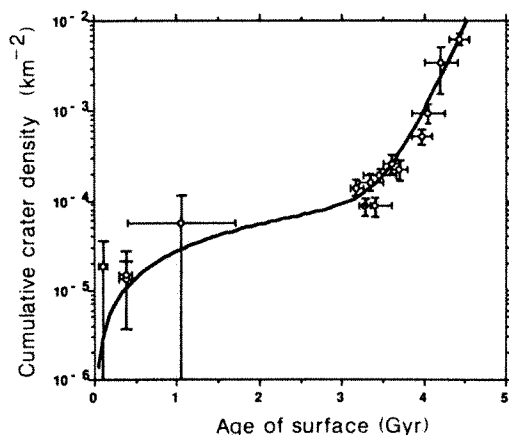


FIG. 2 Comparison between the lunar impact crater density and that predicted by the flux model of equation (5). The revised Schmidt-Holsapple scaling relations for silicate projectiles<sup>9</sup> were used to equate the flux to a crater density assuming an average impact velocity of 20 km s<sup>-1</sup>, mean impact angle of 45°, and a rim-to-rim diameter 25% larger than the apparent transient crater diameter. This yields a cumulative crater density for craters  $> 4$  km diameter of  $N(> 4 \text{ km}) = 2.68 \times 10^5 [T + 4.57 \times 10^{-7} (e^{\lambda T} - 1)]$ , where  $T$  is the age of the cratered surface and  $\lambda = 4.53 \text{ Gyr}^{-1}$ . Crater density data from the compilation in ref. 7, Table 8.4.2.

estimated to between one and four times the lunar rate, with a preferred mean of about two (ref. 7).

Using this constant cratering rate, the rate of mass loss from a planet's atmosphere  $dM_{\text{atm}}/dt$  is given by the flux  $N_{\text{cum}}(m_*)$  of projectiles large enough to remove the atmosphere above the tangent plane, multiplied by the planet's surface area  $4\pi R^2$ , multiplied by the mass above the tangent plane  $m_{\text{tp}} = M_{\text{atm}}(H/2R)$  for  $R \gg H$ . As  $m_*$  itself depends upon  $M_{\text{atm}}$ , a simple differential equation for  $M_{\text{atm}}$  (or, equivalently,  $P$ ) results, whose solution, converted to a convenient and universal form, is:

$$\frac{M_{\text{atm}}(t)}{M_0} = \frac{P(t)}{P_0} = \left(1 - \frac{t}{t_*}\right)^{1/b} \quad (3)$$

where  $M_0$  is the present ( $t=0$ ) atmospheric mass,  $P_0$  is the present atmospheric pressure and  $t_*$  is the length of time required for impacts to reduce the atmospheric pressure to zero. In terms of previously defined quantities,

$$t_* = \frac{1}{2\pi ab(RH)^{1-b}} \left(\frac{2\pi P_0}{g}\right)^b \quad (4)$$

Note that the above equation for the time dependence of atmospheric mass or pressure can be used for times before the present ( $t < 0$ ), so that the atmospheric pressure at any previous era can also be computed. It is interesting to note that the atmospheric pressure declines rigorously to zero at time  $t_*$ —it does not simply fall exponentially towards zero. This interesting fact (K. Zahnle, personal communication) is a unique characteristic of the impact erosion mechanism. As the atmospheric pressure declines, smaller projectiles are capable of removing the atmosphere above the tangent plane. But by the distribution law, there are more smaller projectiles than larger ones, so a greater fraction of the atmosphere is removed in each unit time interval. The net result is the complete stripping of the atmosphere after time  $t_*$ , barring volcanic or other sources of replenishment.

The post-late-heavy-bombardment flux, along with other parameters, gives a value of  $t_* \approx 60$  Gyr for Mars at the present time (note that  $t_*$  for the Earth and Venus is longer by several orders of magnitude, so that the impact erosion of their atmospheres is entirely negligible). Although this number is quite uncertain, it should probably be regarded as an upper limit, as the enhancement of vaporization by oblique impact and a possible large admixture of cometary impacts in the martian cratering flux both tend to shorten  $t_*$ . The adopted value for  $t_*$  predicts an atmospheric pressure at the end of late heavy bombardment (3.2 Gyr) only 1.1 times the present low pressure. This would not explain the geomorphic evidence<sup>12</sup> for running water on Mars's surface early in its geological history. It is widely known<sup>7</sup>, however, that the impact flux on the Moon in the first  $10^9$  years

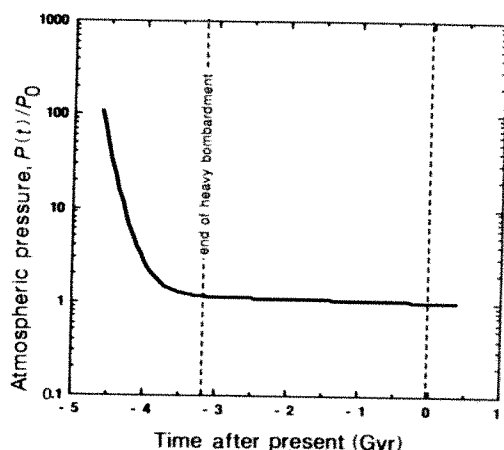


FIG. 3 Evolution of the atmospheric pressure  $P(t)$  on Mars relative to its present value  $P_0$ . This figure is constructed using equation (6) assuming  $t_* = 63.1$  Gyr,  $B = 2,300$  and  $\lambda = 4.53 \text{ Gyr}^{-1}$ . It shows the rapid decline in Mars's atmospheric pressure throughout the era of late heavy bombardment.

of its history was many orders of magnitude larger than at present. It is presumed<sup>11</sup> that Mars, too, shared in this era of 'heavy bombardment', so that the enhanced impact fluxes of this era may have produced an early period of rapid atmospheric-pressure change. The heavy bombardment flux can be adequately described by

$$N_{\text{cum}}(m, t) = a(1 + B e^{-\lambda(t+4.6)})m^{-b} \quad (5)$$

where  $a$  and  $b$  are the same as before,  $B = 2,300$  and  $\lambda = 4.53 \text{ Gyr}^{-1}$ . Figure 2 shows that the crater density computed by this expression provides an excellent fit to the lunar data as far back as 4.4 Gyr.

The differential equation for the rate of atmospheric mass loss implied by equation (5) can be readily integrated, giving a simple analytic expression similar to equation (3) for the evolution of atmospheric pressure as a function of time  $t$  from the present:

$$\frac{M_{\text{atm}}(t)}{M_0} = \frac{P(t)}{P_0} = \left(1 - \frac{t}{t_*} - \frac{B e^{-4.6\lambda}}{\lambda t_*} [1 - e^{-\lambda t}]\right)^{1/b} \quad (6)$$

The atmospheric pressure of Mars predicted by this expression is shown in Fig. 3. It is clear that early in Mars's history its atmospheric pressure must have been at least 100 times its present value, that is,  $\sim 1$  bar. Note that this pressure is near that considered necessary for Mars to have supported free water on its surface<sup>13</sup>.

The simple analytical model of atmospheric erosion described here clarifies several aspects of martian climatic history. First, it is important to emphasize that the present atmosphere of Mars is stable against atmospheric erosion on the timescale of several tens of Gyr. Second, the impact flux during the era of late heavy bombardment was so high that Mars must have had a primordial atmospheric pressure near 1 bar: otherwise, its present atmospheric pressure would be even lower than it is now. This observation agrees well with the geomorphic evidence for fluvial processes during the era of heavy bombardment and with the more indirect evidence<sup>14</sup> for an early era of enhanced erosion that erased small craters and obliterated the rims and other details of larger ones. Mars was able to accumulate its primordial atmosphere in the first place because impact velocities during accretion were too low for the vapour plumes to reach escape velocity. Note that impact erosion does not fractionate atmospheric constituents: a sufficiently large, fast impact removes the atmosphere completely, preserving the ratios of gaseous species while reducing their absolute abundance. However, a condensable substance such as water might be preserved on the surface while other atmospheric constituents are ejected. This property of impact erosion may go far in explaining some of the apparent differences in the atmospheric rare-gas inventories of Mars and the Earth. Our model makes it clear that of all the terrestrial planets, Mars is peculiarly susceptible to impact erosion because of its small radius and low gravitational acceleration. Application of the same model to the Earth indicates a primordial atmospheric pressure about 6 times its present value, whereas Venus would have had an initial atmosphere of only  $\sim 1.5$  times its present pressure.  $\square$

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# Relation between increasing methane and the presence of ice clouds at the mesopause

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**TRENDS** of increasing atmospheric methane, carbon dioxide and other species have now been identified<sup>1</sup>. It is well-known that water vapour is an important product of methane oxidation in the stratosphere<sup>2,3</sup> and here we investigate the possibility that a substantial change has occurred in middle-atmospheric water vapour as a result of the increase in methane over the past century and a half. We show from modelling of mesospheric ice-particle formation that noctilucent cloud brightness should be a sensitive indicator of the water content at the high-latitude summertime mesopause (at a height of 85 km). Blake and Rowland<sup>4</sup> have recently suggested that the occurrence of polar stratospheric clouds may be increasing because of increasing methane. We look at the record of noctilucent cloud occurrence for which the historical record is more complete. We find that noctilucent clouds are absent from the historical record before 1885, which is consistent with our hypothesis.

The hypothesis that upper atmospheric water vapour is increasing because of increases in methane is testable by the consequent effect on noctilucent-cloud (NLC) brightness, which has been recorded for over a century. Our model<sup>5</sup> of NLC ice-particle formation and growth shows quantitatively that cloud brightness may indeed be a sensitive indicator of water-vapour concentration. In view of this, it is remarkable that, despite the fact that observations of NLCs have been made almost every year since their discovery in 1885, none were observed before this date, notwithstanding the attention of a number of skilled observers, located at the appropriate latitudes, who were sufficiently familiar with twilight phenomena. Using our NLC model, we show that clouds were probably not detect-

able in previous centuries because of their weakness compared with the twilight background. Alternatively, however, the discovery of NLCs may have been prompted by the Krakatoa volcanic eruption two years before, which would have caused volcanic injection of water into the stratosphere, followed by slow upward transport to the summertime mesopause. Nevertheless, we expect that the principal cause of most modern NLCs is the current high level of methane-derived water vapour.

Evidence that pre-industrial methane levels were about one half that of the present comes from measurements of reduced methane concentrations within air bubbles trapped in polar ice<sup>6</sup>. Biological sources of methane include anaerobic bacterial fermentation in rice fields, natural wetlands, landfills, domesticated animals (ruminants), and termites. Non-biological sources are natural-gas leaks and venting, mining activities, and industrial activities of several types. The major sink of atmospheric methane is the reaction with the hydroxyl radical (OH). Although the methane budget is not well understood<sup>7</sup>, the globally rising trend is probably a consequence both of increasing source strength (because of increased land use and increasing human and animal populations) and of a global reduction in atmospheric OH levels<sup>8</sup>.

Methane is nearly fully mixed in the troposphere because of its long lifetime (~8 years)<sup>7</sup>. Tropospheric gases are believed to enter the stratosphere in the tropics<sup>9</sup>, although important details of the processes involved are not yet understood<sup>1</sup>. Distribution of methane throughout the stratosphere and mesosphere takes about 2–3 years (ref. 10).

At present, methane oxidation accounts for about one half of the total water above the tropopause<sup>11</sup>. We define total hydrogen as  $[H]_t = [H_2O] + 2[CH_4] + [H_2]$ , where the bracket  $[ ]$  denotes mole fraction or mixing ratio. As there are no sources or sinks of  $[H]_t$  in the upper stratosphere and mesosphere (height of 30–90 km), it is a conserved quantity throughout this region. Thus it is possible to infer changes in water vapour at the high-latitude summer mesopause without the use of a detailed model of atmospheric transport.

Stratospheric methane is slowly removed by complex reactions with OH and with excited atomic oxygen,  $O(^1D)$ , resulting in the production of water, molecular hydrogen and negligible amounts of odd-hydrogen radicals<sup>12</sup>. The expectation that roughly two  $H_2O$  molecules are produced for every  $CH_4$  molecule destroyed has been confirmed by satellite observations of water vapour and methane<sup>11</sup>. Methane oxidation occurs mainly above 30 km, and repartitions  $[H]_t$  primarily into  $H_2O$

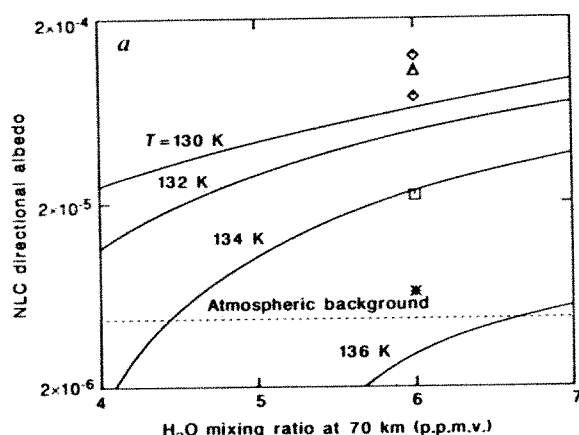
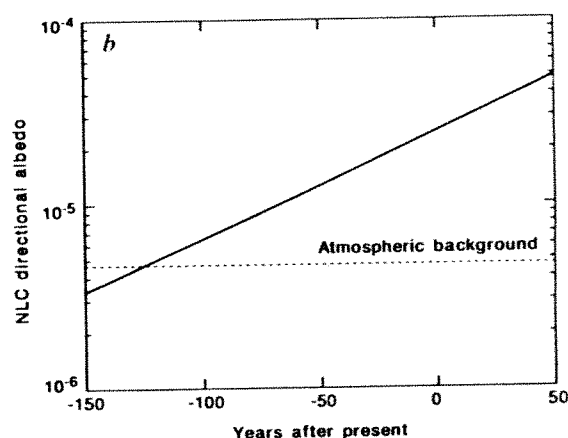


FIG. 1 a, Noctilucent cloud brightness versus water vapour mixing at the lower boundary of the microphysical model. Brightness is measured in terms of the directional albedo<sup>31</sup>, that is, the apparent emission rate divided by the solar flux at a wavelength of 555 nm. The elevation angle of observation and the solar depression angle are both assumed to be 11°. The four curves apply to different values of the mesopause temperature minimum:  $T_m = 130, 132, 134$  and  $136$  K. The Rayleigh-scattered background albedo corresponds



to the viewing conditions assumed in the NLC albedo calculations. Also shown are measurements of the current NLC brightness ( $\Delta$  ref. 32;  $\diamond$  ref. 33;  $\square$  ref. 34; \* ref. 35). b, Noctilucent cloud brightness versus time, in years relative to the present. The values of water vapour were taken from Table 1, and the corresponding values of cloud brightness were taken from the 134-K curve of a.



and  $H_2$ . In the high-latitude summer,  $[H]_t$  consists almost entirely of  $H_2O$ , at concentrations of about 6 p.p.m. by volume  $p$  to about 70 km. A pronounced vertical gradient of  $[H_2O]$  occurs in the upper mesosphere through the progressive photolytic destruction of  $H_2O$  (ref. 13). In this region,  $H_2$  is formed from photolytic reactions among the products of water-vapour dissociation, which consist of odd-hydrogen free radicals. At the high-latitude summer mesopause, the partitioning of  $H_2O$  and  $H_2$  is predicted<sup>12</sup> to be about equal, at 2 p.p.m.v.

With a current atmospheric mixing ratio of  $\sim 1.65$  p.p.m.v.,  $CH_4$  contributes about 55% of the total hydrogen<sup>11</sup>. The remaining 2.7 p.p.m.v. (or 45%) is derived from tropospheric/oceanic sources of  $H_2O$ . Assuming that the latter sources are constant in time, we may calculate the time variation of the water-vapour mixing ratio at 70 km that results from increasing methane levels. Table 1 shows the results calculated using the methane levels reported from ice bubble analyses<sup>6</sup>. As recently as 100 years ago, the  $H_2O$  level may have been only 4.5 p.p.m.v., as opposed to the current value of 6 p.p.m.v.

The current levels of  $H_2O$ —6 p.p.m.v. at 70 km and  $\sim 2$  p.p.m.v. at the high-latitude summer mesopause—are sufficient to explain NLC brightness on the basis of particle growth modelling<sup>14</sup>. It is necessary that the mesopause temperature,  $T_M$ , be less than 135 K so that supersaturation conditions are met at these very low humidities<sup>15</sup>. Water-ice particles are nucleated near the mesopause by meteoric dust and/or by heavy-water cluster ions<sup>14</sup>. Several factors control the NLC brightness in our one-dimensional model, namely temperature and pressure at the mesopause, water-vapour mixing ratio, vertical diffusion rate, and vertical wind speed<sup>5</sup>. Unfortunately, none of these properties is well-known, and in addition they are probably variable from day to day. They must be treated as parameters in the model. Most relevant here is the dependence of cloud brightness on amount of water vapour and on mesopause temperature.

Shown in Fig. 1a are the model calculations of maximum NLC albedo plotted for a range of water-vapour mixing ratios at the lower boundary height of 70 km. We calculate  $H_2O$  concentrations above the lower boundary height by a combination of advective and diffusive transport, photolysis, and sublimation onto and from ice particles. The various curves correspond to different values of  $T_M$ , selected to fall within the observed range. Note that  $[H_2O]$  at the observed height of NLCs (83 km) will be appreciably less than 6 p.p.m.v., because of solar photodissociation and depletion by growing ice particles. Also indicated in Fig. 1a is the range of NLC albedos reported in the literature.

For fixed  $T_M$  the model cloud brightness is very sensitive to  $[H_2O]$ , changing by more than an order of magnitude over a small range in humidity. This sensitivity is caused by the increase in particle growth rate, and resulting maximum particle size, with increasing  $H_2O$  concentration. The scattered radiance depends strongly on the effective particle radius  $r$  (being proportional to  $r^\alpha$ , with  $\alpha \approx 5-6$ ). These factors combine to yield a cloud radiance that scales as  $[H_2O]^\beta$ , with  $\beta \approx 2-3$  (ref. 5). However, this scaling law was derived by assuming a fixed number of cloud particles,  $n$ . In our model, we use a nucleation scheme<sup>14</sup> in which ice deposition occurs only on dust particles whose radius exceeds a critical value. For a fixed temperature, this critical radius decreases with increasing  $H_2O$  concentration. Because the concentration of dust particles increases sharply with decreasing radius, a small increase in  $H_2O$  concentration results in a large increase in the number of particles nucleated. The combined effects of increasing both the numbers and the sizes of cloud particles explains the strong sensitivity of NLC brightness to  $[H_2O]$ . This new model yields values of the exponent,  $\beta$ , that vary between 4 and 8, depending on various model parameters.

Based on the trend of  $[H_2O]$  in Table 1, the evolution of NLC brightness with time is shown in Fig. 1b, along with the calculated Rayleigh-scattered background albedo. We show the results for

$T_M = 134$  K only, but the same general behaviour would be seen at other temperatures.

We now ask whether NLCs were likely to have been present but unnoticed in earlier years. The first observation of NLCs was made by Backhouse<sup>16</sup> on 8 June 1885 in Bad Kissingen, Germany. Some days later this phenomenon came to the attention of numerous astronomers and meteorologists throughout Europe, Great Britain<sup>17</sup> and in Russia<sup>18</sup>. Certain individuals confessed explicitly to have never seen NLCs before this date<sup>19-27</sup>. Some reports of twilight enhancements before 1885 may be attributed to the phenomenon of 'bright nights', that is, enhanced airglow emission, which has been seen at all latitudes and seasons.

Evidence that NLCs were not observed before 1885 is not conclusive. If, however, a pre-1885 sighting of NLCs were to be confirmed, our hypothesis would not necessarily be damaged; we argue only that such sightings were much less likely than they are today. In fact, we believe that the very first sighting was probably not due to the slow emergence of NLCs above the sky background, but that the upper atmosphere probably received an added 'boost' of condensation nuclei and/or water vapour from the Krakatoa volcanic eruption in 1883. The two-year time delay is consistent with the time needed to transport material from the stratosphere to the upper mesosphere<sup>28</sup>, and has been verified by recent model calculations<sup>10</sup>. From Fig. 1a, an abrupt enhancement in NLC brightness would require an injection of 1-2 p.p.m.v. of  $H_2O$ . Assuming that the water was injected at the observed height of the Krakatoa dust plume, a 1-2 p.p.m.v. increase corresponds to a total additional water mass of 100-200 megatons. (The mass of sulphate injected into the stratosphere by Krakatoa has been estimated to be 100 megatons<sup>36,37</sup>.)

We believe that most of the NLC sightings over the past century can be ascribed to the increased water levels arising from methane oxidation. The periodic rise and fall of NLC activity on shorter timescales (decades) is probably due to shorter-term fluctuations in the controlling factors, the most important being material supplied by volcanic injections<sup>29</sup>.

A direct and important inter-relationship between methane, water vapour and the existence of ice clouds in the middle atmosphere is plausible, but what are the long-term implications? In Table 1 and Fig. 1b we have extrapolated forward in time the curves representing water vapour and NLC brightness. We expect brighter and more widespread displays with longer seasons of occurrence. In fact, Gadsden<sup>30</sup> suggests, on the basis of observations made over the last three decades, that both NLC occurrence frequency and the duration of the NLC 'season' have increased. However, the trend that we would predict over this time interval is much less dramatic than Gadsden's. Furthermore, the time series used in ref. 30, derived from observations in northern Europe, does not agree well with corresponding

TABLE 1 Estimated time history of tropospheric methane and high-latitude mesospheric water vapour

Years (before present)	Methane surface concentration (p.p.m.v.)	Water vapour at 70 km (p.p.m.v.)
150	0.8	4.3
100	0.9	4.3
100	0.9	4.5
50	1.2	5.2
0	1.65	6.0
-50	2.7	8.1

The trends of methane up to the present time is taken from Fig. 1 of ref. 6. Mesospheric water vapour is assumed to originate from the atmospheric/oceanic source of 2.7 p.p.m.v. plus a source from methane oxidation (one  $CH_4$  molecule produces two  $H_2O$  molecules). At 70 km, total hydrogen  $[H]_t$  is assumed to be primarily in the form of  $[H_2O]$ . The future trend for methane is taken to be 1.3% per year.

data from the USSR, so that the reported trend may be of regional but not global significance.

It is unlikely that the current atmospheric structure and dynamics will remain unchanged in the face of the important compositional changes taking place throughout the atmosphere. In particular, reductions in ozone and increases in carbon dioxide will alter both the thermal and dynamic structure of the entire middle atmosphere. As noctilucent clouds are probably under strong dynamic control, even small perturbations could be important.

We conclude the following: (1) the first manifestation of the noctilucent cloud phenomenon occurred in the summer of 1885

in northern Europe; (2) before 1885 there was insufficient water vapour routinely available at the mesopause to form visible clouds; (3) the NLC sightings during the period 1885–1895 were probably made possible in part by the large stratospheric injections of water vapour that resulted from the Krakatoa eruption in 1883; (4) the increase in water from methane oxidation will continue in the immediate future, increasing the occurrence frequency and brightness of NLC; and (5) in the long term, the continued rise of trace substances in the atmosphere will act to change the structure and transport within the middle atmosphere in ways that are extremely difficult to predict, making the future of NLC behaviour uncertain. □

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## Limitations on the rapid reduction of nitrogen oxides in exhaust gas streams

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ALL commercially important combustion systems produce exhaust gases containing substantial amounts of nitrogen oxides and molecular oxygen. Because of the part played by nitrogen oxides in smog formation and acid-rain production, methods of reducing  $\text{NO}_x$  emissions have been subject to intense research. In 1986, Perry and Siebers<sup>1</sup> proposed a new chemical method for removing  $\text{NO}_x$  from diesel exhaust gas by adding cyanuric acid ( $\text{HNCO}$ )<sub>3</sub>, and suggested that this new process, called RAPRENOx (rapid reduction of  $\text{NO}_x$ ) could control the emission of  $\text{NO}_x$  from most combustion devices. Here we show that surface chemistry is responsible for the effective reduction of  $\text{NO}_x$ , confirming recent results<sup>2</sup>. In stainless steel, in the absence of oxygen,  $\text{NO}$  is effectively reduced by cyanuric acid at 700 °C. When oxygen is present, however,  $\text{NO}$  is produced by the reaction with cyanuric acid at 700 °C. Under conditions where surface effects are important (such as in the first demonstration experiments reported on RAPRENOx) the reduction of  $\text{NO}$  by cyanuric acid is adversely affected by oxygen.

To learn more about the chemistry involved in RAPRENOx, we carried out a series of laboratory flow-tube experiments under conditions similar to those reported initially<sup>1</sup>. We used two different flow tubes, one quartz and the other stainless steel; except for this difference in material, the two flow tubes were identical (22 mm inner diameter, 80 cm long). Pure gases ( $\text{Ar}$ ,  $\text{NO}$ ,  $\text{CO}$ ,  $\text{CO}_2$ ,  $\text{O}_2$ ,  $\text{H}_2$ ,  $\text{N}_2$  and  $\text{N}_2\text{O}$ ) were admitted to the flow tube through calibrated electronic flow controllers. The flow tube has two independently controlled heated sections: a pre-

heater section (20 cm long) and a reaction section (25 cm long) immediately downstream. Cyanuric acid was sublimed from a quartz crucible lowered into the centre of the preheater region; adjusting the preheater temperature provided independent control of the cyanuric acid sublimation rate.

A mass spectrometer sampled the gas flow downstream of the reaction region. The mass spectrometer sensitivity for the gases of interest was measured before and after each experiment using known flows of the pure gases. Water was also observed in these experiments; because of problems related to  $\text{H}_2\text{O}$  condensation on the flow tube walls, no attempt was made to measure  $\text{H}_2\text{O}$  quantitatively.  $\text{Ar}$  was the predominant gas (>95%). Typical conditions were: pressure 28 kPa; reactor temperature 700 °C; residence time at 700 °C = 1 s. These conditions were chosen to approximate those of the earlier study<sup>1</sup>.

In the quartz flow tube, most of the cyanuric acid which sublimed in the preheater region deposited out onto the cool flow-tube walls downstream of the 700 °C reaction. A small fraction of the cyanuric acid did decompose; the observed decomposition products included  $\text{HNCO}$ ,  $\text{CO}$ ,  $\text{CO}_2$ ,  $\text{N}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{NH}_3$  and  $\text{HCN}$ . Addition of  $\text{NO}$  upstream of the cyanuric acid produced no change in the thermal decomposition of cyanuric acid, and no reduction of  $\text{NO}$  was observed.

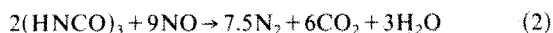
In the otherwise equivalent stainless-steel flow tube, cyanuric acid decomposed rapidly at 700 °C; no solid deposits were observed on the flow-tube walls. The decomposition products were  $\text{CO}$ ,  $\text{H}_2$  and  $\text{N}_2$ , and a small amount of  $\text{CO}_2$ . We analysed the mass spectrometer data and determined the overall stoichiometry of this fast thermal decomposition in stainless steel as:



The small amount of  $\text{CO}_2$  observed results at least in part from the oxidation of  $\text{CO}$  on the walls of the heated flow tube. The independently measured sublimation of cyanuric acid agreed well with the amounts of H, N and C measured in  $\text{H}_2$ ,  $\text{N}_2$  and  $\text{CO} + \text{CO}_2$ .

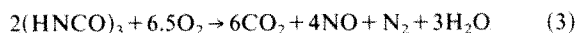
In stainless steel at 700 °C, we observed the rapid reduction

of NO by cyanuric acid. The chemistry is represented by the following stoichiometry:



All species were measured independently except  $\text{H}_2\text{O}$ . No  $\text{N}_2\text{O}$  was observed. The 1-s residence time at  $700^\circ\text{C}$  was sufficient for complete reaction. These observations of the importance of surface chemistry confirm recent findings<sup>2</sup>. Experiments to identify the role of the surface interactions are in progress.

We also find that cyanuric acid reacts rapidly with  $\text{O}_2$  at  $700^\circ\text{C}$  in stainless steel. Our quantitative measurements are consistent with the following overall stoichiometry:



This does not represent the molecular kinetics, of course; it is simply a balanced relationship between major reactants fed into the reactor and major products observed downstream. NO is the major nitrogen-containing product.

The competition for limiting cyanuric acid between NO and  $\text{O}_2$  is illustrated by the sequence of mass spectra shown in Fig. 1. The top mass spectrum is for gas flow of 5.0 standard  $\text{cm}^3 \text{min}^{-1}$  of NO and 2.0 standard  $\text{cm}^3 \text{min}^{-1}$   $\text{O}_2$  in argon at  $700^\circ\text{C}$  in stainless steel. The centre mass spectrum shows the downstream species observed upon addition of 5.0 standard  $\text{cm}^3 \text{min}^{-1}$  NO to limiting cyanuric acid sublimation; note the excess NO at 30 AMU. The bottom mass spectrum shows the addition of 2.0 standard  $\text{cm}^3 \text{min}^{-1}$   $\text{O}_2$  to this NO/limiting cyanuric acid flow. Oxygen is consumed (32 AMU) and more NO is observed than was input (top). In limiting cyanuric acid,  $\text{O}_2$  reacts more rapidly than NO under these conditions.

This seems to contradict the original diesel experiment which purported to demonstrate that RAPRENOx was effective in a stainless-steel system in the presence of excess oxygen<sup>1</sup>. We believe, however, that the diesel data support the  $\text{O}_2$  sensitivity

we observe. In the diesel experiment, NO increased by a factor of at least 25 as the cyanuric acid bed temperature was raised to  $\sim 340^\circ\text{C}$ ; further increase in the acid-bed temperature produced a rapid decrease of NO. As the acid-bed temperature was raised, cyanuric acid sublimed more rapidly and more reactant was entrained into the gas stream. Starting with limiting cyanuric acid in the oxygen-containing exhaust stream, NO was produced according to reaction (3); increasing the cyanuric-acid sublimation rate produced more NO by reaction (3) until the oxygen (which could not be detected by the Fourier transform infrared technique used<sup>1</sup>) was consumed. Subsequent further increase in the cyanuric acid sublimation rate led to a decrease in the total NO by reaction (2).

For the conditions we have examined and the conditions originally reported in support of RAPRENOx<sup>1</sup>, surface effects are responsible for rapid reduction of NO by cyanuric acid. Under these conditions, however, NO is produced when  $\text{O}_2$  is present. D. L. Siebers and J. A. Caton (personal communication), as well as Heap *et al.*<sup>2</sup>, have reported NO reduction using cyanuric acid under conditions where the surface effects examined here are not important. This gas-phase NO reduction chemistry is sensitive to temperature and the presence of other gases in the exhaust (ref. 2; D. L. Siebers and J. A. Caton, personal communication). The use of the RAPRENOx technique to control  $\text{NO}_x$  emissions seems therefore to be considerably more limited than was suggested originally<sup>1</sup>. □

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## Glacial–Holocene salinity changes in the Mediterranean Sea: hydrographic and depositional effects

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OXYGEN isotope changes as recorded by Pleistocene foraminifera from open-ocean sediments are primarily a function of changes in the isotopic composition of the global seawater reservoir caused by ice-volume changes, with a secondary temperature component<sup>1</sup>. Oxygen isotope records from marginal basins such as the Gulf of Mexico<sup>2</sup>, the Mediterranean Sea<sup>3–5</sup> and the Red Sea<sup>6</sup> also exhibit the global signal but are far more complicated owing to the strong overprint of local climate conditions. In particular, glacial–interglacial changes in continental aridity and river runoff produce significant salinity changes in these semi-enclosed basins. Using available oxygen isotope records we estimate that glacial (18,000 yr BP) salinities in the eastern and western Mediterranean Sea were 2.7‰ and 1.2‰ higher, respectively, than at present. These elevated glacial salinities played an important part in preconditioning the eastern Mediterranean for the eventual accumulation of organic carbon-rich sediments (sapropels) at the end of deglaciation.

Our database consists of thirty published planktonic foraminiferal oxygen isotope records from the eastern and western basins of the Mediterranean (Table 1; refs 7–17). For each record, the magnitude of the oxygen isotope change has been estimated for three different intervals: (1) from 18,000 yr BP to present, (2) from 18,000 yr BP to 8,000 yr BP, and (3) from 8,000 yr BP to present (Table 1). The 8,000 yr BP level represents

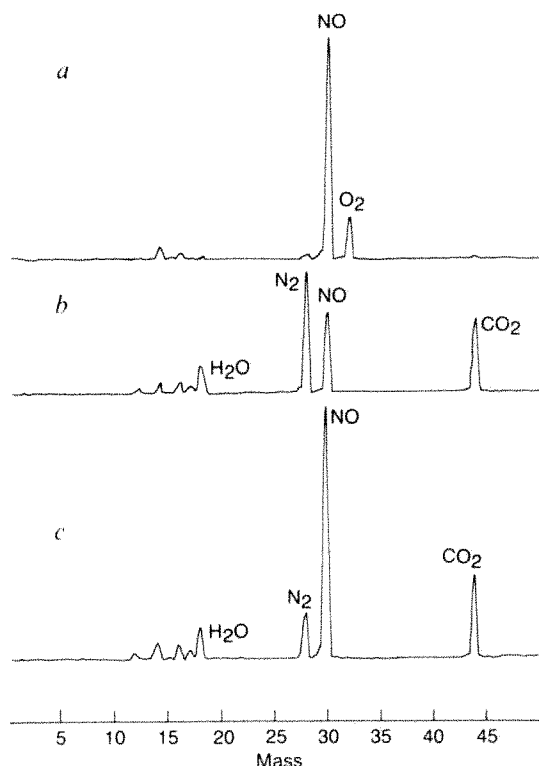


FIG. 1 Mass spectra showing the competition for limiting cyanuric acid between NO and  $\text{O}_2$  at  $700^\circ\text{C}$  in stainless steel. a, NO = 5.0 standard  $\text{cm}^3 \text{min}^{-1}$ ,  $\text{O}_2$  = 2.0 standard  $\text{cm}^3 \text{min}^{-1}$ , no cyanuric acid sublimation. b, NO = 5.0 standard  $\text{cm}^3 \text{min}^{-1}$ , no  $\text{O}_2$ , and stable limiting cyanuric acid sublimation. c, Same as b, to which 2.0 standard  $\text{cm}^3 \text{min}^{-1}$   $\text{O}_2$  has been added.



the midpoint (in time) of deposition of the most recent sapropel<sup>8</sup>. For each of these time intervals we estimate how much of the observed isotopic change can be attributed to salinity change ( $\Delta S$ ) using the following equations:

$$\Delta S_1 = (\Delta 18k-P) - V - \Delta T_1 \quad (1)$$

$$\Delta S_2 = (\Delta 18k-8k) - V - \Delta T_2 \quad (2)$$

$$\Delta S_3 = (\Delta 8k-P) - V - \Delta T_3 \quad (3)$$

where  $\Delta S_1$ ,  $\Delta S_2$  and  $\Delta S_3$  are  $\delta^{18}O$  changes (‰) owing to salinity changes for the intervals 18,000 yr BP-present, 18,000 yr BP-8,000 yr BP and 8,000 yr BP-present, respectively.  $\Delta 18k-P$ ,  $\Delta 18k-8k$  and  $\Delta 8k-P$  are the calculated  $\delta^{18}O$  changes for the three time intervals and  $V$  is the glacial-interglacial ice volume contribution to the  $\delta^{18}O$  changes.  $\Delta T_1$ ,  $\Delta T_2$  and  $\Delta T_3$  are the  $\delta^{18}O$  changes attributable to temperature for the three time intervals.

To solve these equations we have assumed that deglaciation was virtually complete by 7,000-8,000 yr BP (refs 19, 20). Therefore, the ice-volume ( $V$ ) contribution to the total  $\delta^{18}O$  change in equations (1) and (2) is assumed to be the same, and the ice-volume component in equation (3) is assumed to be zero. We have also assumed that the warming of surface-water temperatures from glacial to present-day values in the eastern and western Mediterranean was complete by ~8,000 yr BP. This does not imply that there have been no temperature or climatic oscillations in the Mediterranean region during the last 8,000 yr. In fact temperatures were probably warmer during the thermal maximum at ~6,000 yr BP. What we have assumed is that there has been no significant net change in temperature between 8,000 yr BP and present. This assumption is supported by palaeotemperature estimates for the adjacent eastern North

Atlantic<sup>21</sup>. As a result,  $\Delta T_1 = \Delta T_2$  and  $\Delta T_3 = 0$ . From these assumptions equation (3) becomes simplified to:

$$\Delta S_3 = \Delta 8k-P \quad (4)$$

which means that the entire difference in  $\delta^{18}O$  between 8,000 yr BP and present is assumed to be due to salinity changes.

Using the data in Table 1, mean values have been determined for the isotopic changes associated with the three time intervals of interest for both the eastern and western Mediterranean (Table 2a; Fig. 1). The mean  $\delta^{18}O$  changes between 18,000 yr BP and present are very similar, 3.1‰ and 2.9‰ for the eastern and western basins, respectively. In contrast, the isotopic differences between the eastern and western basins for the other two time intervals are relatively large. The  $\delta^{18}O$  difference between 18,000 and 8,000 yr BP is 0.7‰ greater in the eastern basin (4.3‰) than in the western basin (3.6‰). Similarly, the  $\delta^{18}O$  response between 8,000 yr BP and present is 0.4‰ larger in the eastern basin (-1.2‰) than in the western basin (-0.8‰) (Table 2a).

The salinity contribution to the observed oxygen isotope change between any two data points can be determined if reasonable estimates are available for the ice-volume effect and the magnitude of temperature change. The ice-volume effect for the most recent glacial-interglacial transition was between 1.1 and 1.3‰ (ref. 22). For our calculations, an ice volume ( $V$ ) of 1.2‰ is used. Foraminiferal transfer-function estimates of glacial sea surface temperatures in the Mediterranean<sup>23,24</sup> indicate that the western and eastern basins were on average ~6 °C and 4 °C colder than at present, respectively. Using the  $\delta^{18}O$ -temperature relationship of 0.2‰ per °C (ref. 25) means that 1.2‰ and 0.8‰ of the total glacial-interglacial  $\delta^{18}O$  changes ( $\Delta T_1$  and  $\Delta T_2$ ) in the western and eastern basins are a function of temperature.

TABLE 1 Planktonic foraminiferal oxygen isotope data for Mediterranean cores

Core number	Latitude	Longitude	18k (‰)	8k (‰)	Present (‰)	18k-P (‰)	18k-8k (‰)	8k-P (‰)	Ref.
<b>Western Mediterranean</b>									
82KS32	36°07' N	02°07' W	3.3		0.3	3.0			7
82KS31	36°09' N	03°16' W	3.2		0.5	2.7			7
82KS30	36°27' N	03°53' W	3.2		0.5	2.7			8
C68	35°41' N	04°05' W		-2.0	0.5			-2.5	9
82KS41	35°59' N	04°24' W	2.5		0.5	2.0			8
70KS05	38°06' N	02°59' E	3.2	-1.2	-1.0	4.2	4.4	-0.2	8
70KS06	38°31' N	04°00' E	3.2	-0.8	0.5	2.7	4.0	-1.3	10
C3	42°47' N	07°41' E	4.0	-1.6	-1.0	5.0	5.6	-0.6	11
KET8022	40°35' N	11°42' E	3.9	1.2	1.6	2.3	2.7	-0.4	12
70CS05	35°44' N	13°11' E	3.6	0.5	0.8	2.8	3.1	-0.3	5
KET8004	39°40' N	13°34' E	3.9	1.2	1.4	2.5	2.7	-0.2	12
KET8003	38°49' N	14°29' E	3.8	0.8	1.4	2.4	3.0	-0.6	12
<b>Eastern Mediterranean</b>									
KS09	35°09' N	20°09' E	3.3		-0.8	4.1			13
V10-67	35°42' N	20°43' E	3.0	-1.2	0.5	2.5	4.2	-1.7	8
TR171-24	34°03' N	22°32' E	4.1	1.2	2.5	1.6	2.9	-1.3	3
67M03	34°25' N	24°50' E	4.7	-0.6	1.4	3.3	5.3	-2.0	5
RC9-181	33°25' N	25°00' E	3.1	-1.2	0.1	3.0	4.3	-1.3	13
TR171-27	33°50' N	25°59' E		0.1	1.8			-1.7	3
CHN119-6	33°15' N	26°00' E	3.6	0.2	0.7	2.9	3.4	-0.5	4
75KS52	34°00' N	26°19' E	3.2	-0.8	0.0	3.2	4.0	-0.8	14
75KS50	34°41' N	27°00' E	3.6	-1.8	0.0	3.6	5.4	-1.8	5
ALB189	33°54' N	28°29' E	3.5						13
CHN119-16	33°14' N	30°19' E		-0.2	0.7			-1.0	4
CH119-18	34°20' N	30°53' E	4.6	0.1	0.7	3.9	4.5	-0.6	4
CH119-22	32°46' N	31°53' E		-0.6	0.9			-1.5	4
SH281-17	35°30' N	34°07' E	4.0	-1.1	-0.3	4.3	5.1	-0.8	15
GA32	31°57' N	34°21' E		-2.0	-1.0			-1.0	16
SH190-19	36°00' N	34°22' E	4.3	-0.5			4.8		15
82KS01	34°22' N	27°09' E	3.4		0.0	3.4			17
TR172-22	35°19' N	29°01' E	3.0	0.3	0.9	2.1	3.0	-0.6	3

The salinity component of the observed  $\delta^{18}\text{O}$  changes has been calculated for each of the three time intervals using equations (1), (2) and (4) (Table 2b). To convert the salinity portion of the  $\delta^{18}\text{O}$  change to salinity, we use the modern seawater oxygen-isotope/salinity relationship for the Mediterranean<sup>26</sup>. According to this relationship, a 1‰ change in salinity will result in a 0.41‰ change in seawater  $\delta^{18}\text{O}$ . Using the present-day relationship between seawater oxygen isotopic composition and salinity to estimate past salinities is probably too simple. However, as it is not possible to define such a relationship for the past, using the modern relationship is the most reasonable solution to the problem.

The mean planktonic foraminiferal  $\delta^{18}\text{O}$  changes between the last glacial maximum and the present in the western and eastern basins of the Mediterranean are 2.9‰ and 3.1‰, respectively (Table 2a; Fig. 1). These are considerably larger than the 1.8‰ change recorded in the Atlantic<sup>27</sup>. If 1.2‰ of the mean change is due to ice-volume changes,  $\delta^{18}\text{O}$  residuals of 1.7‰ for the western basin and 1.9‰ for the eastern basin represent the combined effect of temperature and salinity change. An additional 1.2‰ and 0.8‰ of the  $\delta^{18}\text{O}$  residuals for the western and eastern basins are accounted for by glacial sea surface temperatures being 6 °C and 4 °C colder than at present<sup>23,24</sup>. Of the total  $\delta^{18}\text{O}$  changes that are observed between 18,000 yr BP and present, 0.5‰ and 1.1‰ are due to salinity changes in the western and eastern Mediterranean, respectively (Table 2b). These  $\delta^{18}\text{O}$  changes are equivalent to salinity changes of 1.2‰ in the western basin and 2.7‰ in the eastern basin (Table 2b). Typical present-day surface salinities in the western and eastern Mediterranean are 38.2‰ and 38.8‰, respectively. From the oxygen isotope results, we estimate that salinities increased to 39.4‰ in the western basin and 41.5‰ in the eastern basin during the last glacial.

The mean  $\delta^{18}\text{O}$  change between 18,000 yr BP and 8,000 yr BP is 3.6‰ for the western basin and 4.3‰ for the eastern basin (Table 2a, Fig. 1). Under the assumptions that deglaciation and surface-temperature warming were virtually complete by 8,000 yr BP, the salinity contribution to the observed  $\delta^{18}\text{O}$  changes is 1.2‰ for the western basin and 2.3‰ for the eastern basin (Table 2b). These  $\delta^{18}\text{O}$  changes represent salinity changes of 2.9‰ and 5.6‰ for the western and eastern Mediterranean, respectively (Table 2b). Using the salinities determined for 18,000 yr BP, we estimate that salinities at 8,000 yr BP were ~36.5‰ in the western basin and 35.9‰ in the eastern basin.

TABLE 2 Oxygen isotope and salinity changes in the Mediterranean

a. Mean planktonic foraminifera			
Time interval	Western basin*	Eastern basin*	
18,000 yr BP-present	2.9‰	3.1‰	
18,000 yr BP-8,000 yr BP	3.6‰	4.3‰	
8,000 yr BP-present	-0.8‰	-1.2‰	

b. Oxygen isotope changes attributed to salinity and estimated salinity changes ( $\Delta S$ )				
Time interval	Western basin		Eastern basin	
	$\delta^{18}\text{O}$ (‰)	$\Delta S$ (‰)†	$\delta^{18}\text{O}$ (‰)	$\Delta S$ (‰)†
18,000 yr BP-present	0.5	1.2	1.1	2.7
18,000 yr BP-8,000 yr BP	1.2	2.9	2.3	5.6
8,000 yr BP-present	-0.8	-1.9	-1.2	-2.9

\* Based on data in Table 1.

† Derived from the seawater oxygen isotope/salinity relationship for the Mediterranean<sup>26</sup> whereby a 1.0‰ change in salinity produces a 0.41‰ change in  $\delta^{18}\text{O}$ .

As previously discussed, all of the observed  $\delta^{18}\text{O}$  change between 8,000 yr BP and the present can be attributed to salinity. The -0.8‰ and -1.2‰  $\delta^{18}\text{O}$  changes for the western and eastern basins (Table 2b) are equivalent to salinity increases of 1.9‰ in the western basin and 2.9‰ in the eastern basin between 8,000 yr BP and the present.

The estimated salinity changes have a number of important implications for both the palaeohydrographic and depositional conditions in the Mediterranean since the last glacial period. The present arid conditions in the Mediterranean region create a negative water balance (in that evaporation exceeds precipitation and runoff) which results in an anti-estuarine exchange with the North Atlantic (Fig. 2a). Our isotopic results indicate that these conditions changed during the last glacial (Fig. 2c). The higher glacial salinities suggest that the water balance of the Mediterranean became more negative as the region became even more arid than at present. This is in agreement with pollen and lake-level records from the circum-Mediterranean. In particular, African lake levels were at a minimum during the last glacial<sup>28</sup> and pollen spectra indicate colder, drier conditions between 26,000 and 13,000 yr BP than today<sup>29</sup>.

The isotopic evidence suggests that the west-to-east gradient in surface salinities was maintained during the last glacial, implying that surface and deep-water exchanges were similar to those of today (Fig. 2c). The Mediterranean was still exporting dense outflow water to the Atlantic during the last glacial<sup>30</sup>, although the volume of outflow was considerably reduced due to eustatically controlled restriction at the Gibraltar sill<sup>26</sup>. The salinity and density of glacial intermediate and deep waters in the Mediterranean would also have been elevated, because the sources of these water masses are simply Mediterranean surface waters. Because oxygen solubility decreases with increasing salinity, glacial deep waters in the Mediterranean probably had lower dissolved oxygen concentrations than at present.

Hydrographic conditions in the Mediterranean at 8,000 yr BP must have been considerably different than at 18,000 yr BP. The water balance became positive as precipitation and run-off exceeded evaporation (Fig. 2b). Salinities were considerably lower at 8,000 yr BP and the west-east salinity gradient was reversed to an east-west gradient. This is supported by East African climate records which indicate the onset of very humid conditions at ~12,500 yr BP, with wettest conditions occurring between 10,000-8,000 yr BP (refs 14, 28). This was also a time of intensified African monsoons and increased Nile discharge<sup>14,31</sup>.

The reversal of the salinity gradient coupled with a positive water balance in the Mediterranean at 8,000 yr BP strongly suggests a reversal of the surface flow patterns, that is, surface waters flowed out of the Mediterranean (Fig. 2b). This reversal

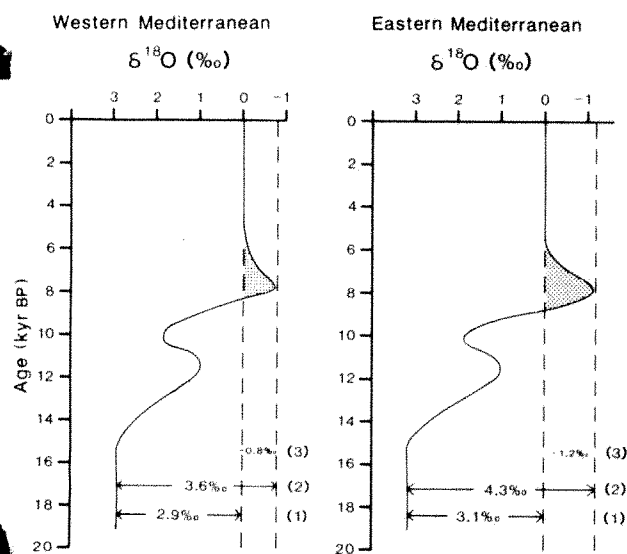


FIG. 1 Generalized planktonic foraminiferal oxygen isotope records for the western and eastern Mediterranean. The magnitudes of the isotopic differences between (1) 18,000 yr BP-present, (2) 18,000 yr BP-8,000 yr BP, and (3) 8,000 yr BP-present are indicated on each record.

of circulation patterns, previously proposed on the basis of sedimentological results<sup>32</sup>, may have begun as early as 12,000 yr BP with the onset of pluvial conditions in this region. Such a circulation change means that the Mediterranean was not a source of dense outflow to the Atlantic at this time.

As previously mentioned, 8,000 yr BP represents the midpoint of the deposition of the most recent sapropel (7,000–9,000 yr BP)<sup>18</sup>. Most workers have invoked versions of two general models for sapropel formation: a 'density-stratification' model and a 'productivity' model. The density-stratification model requires a low-salinity surface layer which inhibits bottom-water oxygen renewal and eventually results in anoxia<sup>33</sup>. The productivity model simply requires a high organic-carbon flux to produce sapropels<sup>18,34</sup>. Jenkins and Williams<sup>4</sup> and Howell and Thunell<sup>35</sup> evaluated both of these models and concluded that both increased productivity and a cessation of bottom-water renewal were required in order for sapropel deposition to occur within a period of several thousand years.

We believe that the key to understanding the formation of the most recent sapropel in the eastern basin is the glacial  $\delta^{18}\text{O}$  patterns in both basins. The  $\delta^{18}\text{O}$  patterns strongly suggest that high salinities (surface and deep water) during the last glacial were an important factor in 'preconditioning' the Mediterranean for sapropel deposition. First, bottom-water oxygen concentrations would have already been reduced because of the decrease in oxygen solubility associated with the increase in salinity. Second, the very high salinities which existed before sapropel formation would have more easily allowed the establishment of a density stratification with the onset of pluvial conditions. In addition, the circulation reversal would have caused the eastern Mediterranean to become a nutrient trap, thus enhancing pro-

ductivity. The combined effects of these conditions explains the most recent sapropel formation in the eastern Mediterranean and the contemporaneous deposition of reduced but not anoxic hemipelagic sediments in the western Mediterranean<sup>36</sup>.

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## Probable modern analogue of Kuroko-type massive sulphide deposits in the Okinawa Trough back-arc basin

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THE volcanogenic massive sulphide deposits of the Hokuroko district of Japan are Miocene metal occurrences which are rich in both Zn and Pb, contain minor Cu and yield noteworthy amounts of Ag and Au (ref. 1). They formed in the Green Tuff belt of Japan in association with the felsic calc-alkaline rocks of a back-arc spreading centre<sup>1</sup>. Until now no modern analogue of this

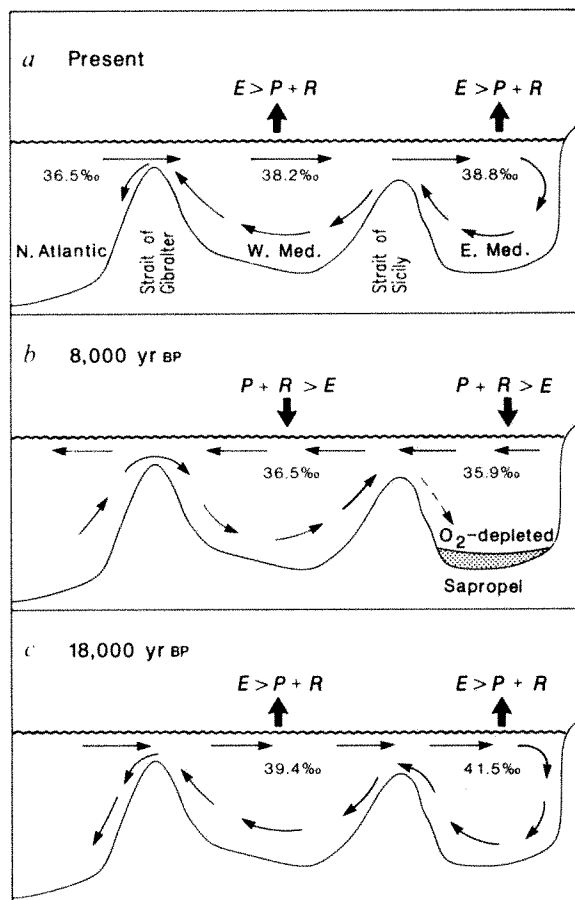


FIG. 2 Cross-sectional views of the Mediterranean showing estimated surface salinities, water balance and inferred circulation patterns for a the present; b, 8,000 yr BP, and c 18,000 yr BP. Here, E, P and R denote evaporation, precipitation and runoff.



'Kuroko' type of sulphide deposit has been identified; this is in contrast to the Cyprus type of massive Cu-Zn sulphide deposit, which has such an analogue in the 'smoker' deposits of the East Pacific Rise. In 1984 and 1986, low-temperature oxide and silicate precipitates were discovered in the rift valley of the Okinawa Trough attesting to the recent occurrence of hydrothermal activity in this intracontinental back-arc basin<sup>2</sup>. On 26 June 1988, a large hydrothermal field with extensive occurrences of sulphide mineralization (the Jade hydrothermal field) was discovered and sampled by the German research vessel *Sonne* in a cauldron in the central part of the Okinawa Trough. Here we report geochemical and isotopic results which suggest that the Jade deposit is a modern analogue of the Kuroko-type deposits known so far only from the geological record.

The Okinawa Trough, located between the Ryukyu island chain and the east coast of China, is an active back-arc basin formed by extension within the continental lithosphere behind the Ryukyu trench-arc system. Evidence for the presence of active back-arc spreading exists only in the southern trough, whereas the main part of the graben system, characterized by normal faulting and crustal extension, is considered to be in the rifting phase<sup>3</sup>. The Middle Okinawa Trough probably represents a transition from present-day arc volcanism to volcanism associated with back-arc spreading. The local sea-floor map shows a series of parallel elongated ridges with 70° ENE strike, whereas the major structural grain is about 35° E which is indicative of a shear component in the deformation of the Middle Okinawa Trough. The ridges are composed of a bimodal assemblage of young volcanic rocks: basalt, andesite, dacite and rhyolite. First analyses made on board show that, according to their major-element geochemistry<sup>4</sup>, all the rock samples are of calc-alkaline island-arc type. The valleys and ridges are cut by normal faulting, giving rise to younger volcanic intrusions. Thus it is evident that the Okinawa Trough is a thermally active area<sup>5,6</sup> in which magma supply systems and extension processes create hydrothermal circulation.

The Jade hydrothermal field was discovered in a caldera-like structure (the Izena Cauldron<sup>7</sup>) which has a diameter of about 5 km. It is located at about 27°15.0' N and 127°04.5' E and lies 36 km south of the deposit from the hydrothermal mounds (Fig. 1a). The central part of the cauldron, which has a maximum water depth of about 1,650 m, is filled with younger, unconsolidated sediments at least 10–20 m thick according to our 3.5-kHz record<sup>4</sup>. Preliminary results show that heat flow at the bottom of the cauldron is high and variable, which indicates that hydrothermal circulation is probably taking place. The measured heat flow ranges from 100 to 800 mW m<sup>-2</sup> and increases somewhat towards the north (Fig. 1b). By following this gradient, we discovered the hydrothermal vent area in the northern part of the inside north-east slope (Fig. 1b) by instrumented deep tow using the Ocean-Floor Observation System. Two photographic, temperature and television profiles were carried out across the vent area; temperature measurements showed anomalies of up to ~0.5 °C (Fig. 1b). The local topography of the Jade field is generally rugged with small step-fault features resulting from the intense tectonics within the inner cauldron slope. Animals typical of the hydrothermal-vent communities on the East Pacific Rise<sup>8</sup>, such as white clams, galatheid crabs and lugworms, as well as white starfish, are observed in our deep-sea pictures.

Distinct signals in the water column can be used to identify active hydrothermal-vent areas. Methane contents ranging from ~5.4 to ~6.2 p.p.m. were measured in water between 1,400 and 1,420 m above the hydrothermal field, at a total depth of 1,480 m. The Mn concentration measured above the vent area in the same depth range is ~2–3 parts per 10<sup>9</sup> (p.p.b.) (measured background values of Mn varied between 0.2 and 0.3 p.p.b.). In the central part of the cauldron basin, concentrations as high as 8.8 p.p.m. CH<sub>4</sub> were measured at a depth of about 1,600 m; the CH<sub>4</sub> background in the surroundings of the cauldron is 0.02–0.05 p.p.m. These high CH<sub>4</sub> and Mn concentrations reveal

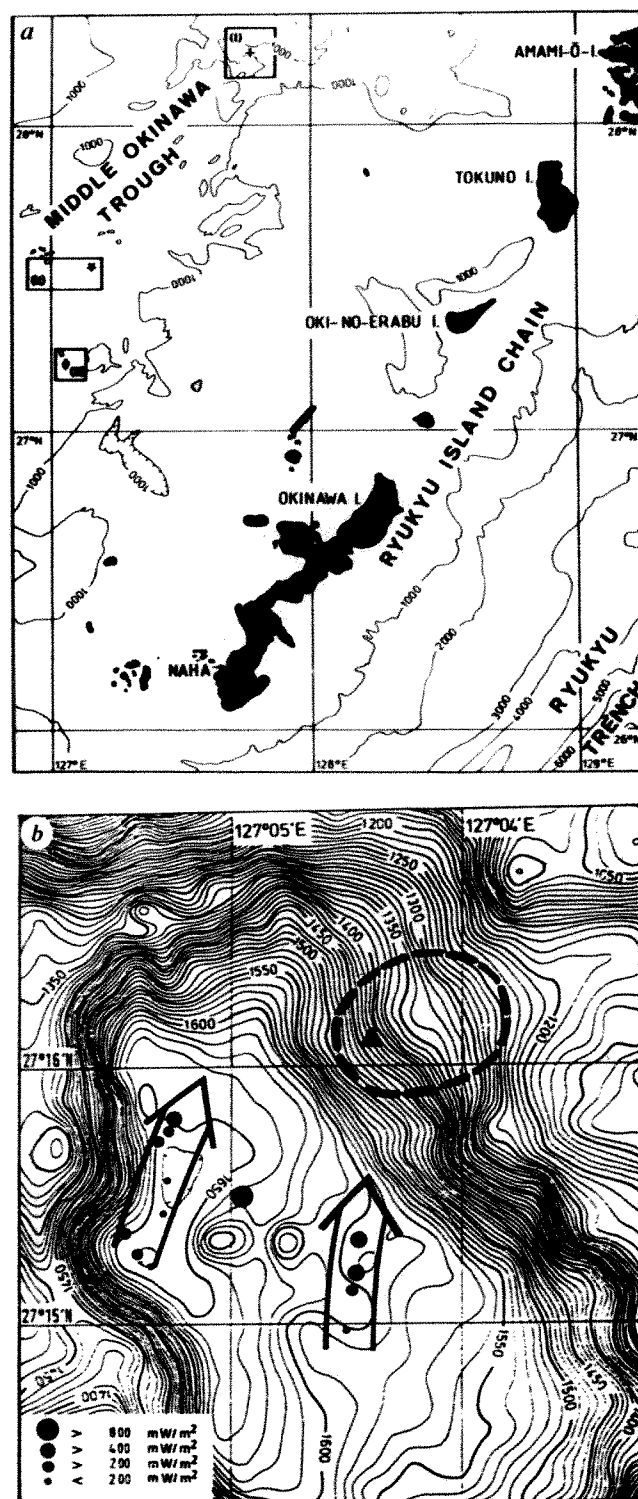


FIG. 1 Location maps for hydrothermal vents in the Okinawa Trough. a, Map<sup>15</sup> of the central part of the Ryukyu trench-arc system, which is located between the island of Kyushu in the north-east and the island of Taiwan in the south-west. A section of the Middle Okinawa Trough is also shown. The areas (I), (II) and (III) were studied during the research cruise S0 56. + and ★ indicate locations of oxidic-siliceous low-temperature hydrothermal-mound deposits, ◆ shows the location of the Izena Cauldron with the Jade Hydrothermal Field. b, Part of the bathymetric map of the Izena Cauldron (location (III) in a). The Jade deposit lies on the inside north-east slope and is delimited by the dashed line. The contour interval of water depth is 10 m. ▲ marks the spot of the temperature anomaly (~0.5 °C) measured about 5 m above the bottom, which indicates hydrothermal venting. The two arrows show the approximate trend of increasing heat flow within the sediments of the basin, based on heat-flow data measured during the S0 56 cruise. The dots indicate heat-flow values.

the existence of a strong hydrothermal plume, which seems to be particularly pronounced because of the protected environment in the caldera.

We identified four different varieties of sea-bed sulphide mineralizations in the dredged material, namely two types of massive sulphides, a stockwork mineralization and a sulphide-bearing sediment layer. We now describe each of these in detail. (1) The first massive sulphide (MS I) comprised black-grey fragments of stacks, which accumulate around and on the hydrothermal vents by direct accretion of minerals precipitated from the hydrothermal fluid. Initial microscopic studies of the ore reveal that they represent a late stage of low-temperature hydrothermal activity. The youngest minerals in the outer layer (0–2 cm thick; Table 1, column A) are barite, realgar, orpiment, amorphous silica and millimetre-thick surface coatings of hydrous iron-manganese oxides. Other sulphide minerals are sphalerite (including schalenblende), Ag-bearing galena and pyrite (Table 1). The central parts (at 4–6 cm depth; Table 1, column C) consist mainly of the three sulphide minerals above; some chalcopyrite is present, but mostly as myrmekitic inclusions or as fine-grained replacement textures described as 'chalcopyrite disease'. The barite and amorphous-silica content is significantly lower than in outer layers, and arsenic minerals are rarer.

(2) The second type of polymetallic sulphide (MS II) is olive-green to grey. The recovered material consists of elongated plates, several centimetres in thickness, which show a very fine and porous structure. The main minerals are sphalerite, chalcopyrite and pyrite, accompanied by anglesite and lesser amounts of amorphous silica. The higher concentration of chalcopyrite and the lack of galena probably indicates a higher primary temperature of formation relative to the MS I type. Anglesite was probably formed at higher oxygen fugacity than were the sulphides. Replacement of previously formed galena by anglesite was not observed.

(3) Within the host rocks of hydrothermally altered rhyolite are found millimetre-thick fracture fillings of stockwork mineralizations. These minerals are sphalerite, tennantite (Ag- and Sb-bearing), galena, enargite and, to a lesser extent, pyrite and chalcopyrite. The altered rhyolite consists of an extremely fine-

grained mixture of quartz, muscovite and some feldspar. Besides the fracture fillings, replacement masses are observed which contain basically the same sulphide minerals but with coarser grain-sizes. In some cracks of both the stockwork mineralization and the MS I type samples, millimetre-thick precipitates of native sulphur occur. Native sulphur is in general an oxidation product of  $H_2S$ . Its occurrence therefore indicates that in the venting hydrothermal fluid the concentration of  $H_2S$  was high and contributed to the formation of native sulphur after precipitation of the prevailing sulphide assemblage.

(4) Within two cores taken from the north-eastern part of the caldera basin, a sulphide-bearing layer of 10–15 cm in thickness was observed at a depth of about 80 cm below the top of the sediment. The fine-grained, unconsolidated, greyish material contains considerable amounts of sphalerite, pyrite and barite; the detrital minerals are quartz, calcite, illite and chlorite. As the sulphide-bearing sediment layer could not be observed in cores from the western part of the caldera basin, we suggest that the sulphide minerals were supplied from the Jade hydrothermal field.

Microthermometric data may be used to estimate mineral formation temperatures and salt concentrations of hydrothermal fluids. Such measurements have been conducted in the outer Ba- and As-rich material (sample A in Table 1) of the MS I type mineralizations. Each of the barite grains was found to be water-clear. The fluid inclusions have average lengths of 5–6  $\mu m$  and are not bound to any cleavage. The nine measurements yielded fairly constant temperatures of homogenization of  $160 \pm 5^\circ C$ . The salt content of the fluid inclusions is somewhat higher than in normal ocean water (freezing temperature  $-1.9^\circ C$ ), as indicated by freezing-point depressions ranging from  $-2.8$  to  $-3.4^\circ C$ . Thus, this barite was formed at about  $160^\circ C$ , probably through mixing of Ba-bearing hydrothermal solution with cold sulphate-rich sea water.

Chemical metal analyses (Table 1) indicate that the Jade massive sulphides are particularly rich in Zn and Pb but contain only minor amounts of Cu, with the exception of samples E (stockwork mineralization) and F (MS II type); samples A to D (MS I type) also exhibit significant Ag and Au content, with the Au/Ag ratio varying between 0.001 and 0.02, which is in the same range as values for other sea-floor massive sulphide deposits<sup>10</sup>. Point measurements, made by electron microprobe analysis, of galena grains in samples B and C revealed an Ag content of  $0.28 \pm 0.06\%$  (32 measurements). Thus, in addition to tennantite, galena is an important host phase of Ag. No Ag was, however, detected in sphalerite.

In comparison with other recent sea-floor sulphide deposits<sup>10,11</sup> the Ag concentration of 0.65% and the Au content of 9.8 p.p.m. (Table 1) are the highest found so far. Concentrations of Ba and As are found to be greatest in the upper 2 cm (Table 1, column A) of the MS I stack samples, indicating that barite, realgar and orpiment formed in the cooler and more oxidizing parts of the hydrothermal system. Au is related to these late precipitates; maximum concentrations of Ag occur more towards the inner parts (Table 1, columns B, C).

Au occurs at minor concentrations (0.3–0.7 p.p.m.; Table 1) in the chalcopyrite-rich samples of the MS II type and stockwork mineralizations, but is enriched by about one order of magnitude in the late paragenetic sulphide-sulphate-silica mineral system. Thus it is reasonable to assume that a remobilization of Au from the earlier, high-temperature sulphides has taken place, followed by reconcentration of Au in the more oxidized outer portions of the deposit through sustained hydrothermal circulation. Late, lower-temperature ( $<250^\circ C$ )<sup>11</sup> solutions ascending through massive sulphides would remove Au as the bisulphide complex  $(Au(HS)_2^-)$  because of the high concentration of sulphur. Mixing with cooler sea water would result in a decrease in the solubility of Au, particularly below  $200^\circ C$  (ref. 10). The chloride complex  $(AuCl_2^-)$  is more important in early, high-temperature ( $300$ – $400^\circ C$ ) solutions<sup>10</sup> and might account for the high Au content

TABLE 1 Metal composition of massive sulphide samples from the Jade Hydrothermal Field (Okinawa Trough)

	A*	B*	C*	D†	E‡	F§
Zn (%)	3.09	35.12	39.89	5.46	15.17	33.26
Cd	<0.01	0.06	0.11	<0.01	0.08	0.13
Pb	10.50	15.85	19.52	8.32	5.91	25.52
Fe	4.81	13.55	11.72	5.63	1.06	7.23
Mn	0.29	0.09	0.10	0.35	0.03	0.08
Cu	0.02	0.34	0.49	0.04	3.64	6.09
SiO <sub>2</sub>	19.36	7.56	1.04	20.63	49.20	2.53
Al	0.10	0.03	0.02	0.02	2.29	0.06
Ca	0.12	0.03	0.03	0.10	0.04	0.03
Mg	0.07	0.03	0.02	0.05	0.10	0.04
Sr	0.21	0.01	0.01	0.25	0.01	0.12
Ba	8.65	0.64	0.05	7.44	0.02	0.03
As	9.31	1.38	0.25	4.40	1.12	0.06
Ag	0.064	0.650	0.410	0.076	0.018	0.050
Au p.p.m.	7.8	9.8	4.8	4.2	0.3	0.7
Au/Ag	0.012	0.002	0.001	0.006	0.002	0.001

Metal determinations of dried substance were carried out by flameless atomic absorption spectrophotometry and inductive coupled plasma methods. All determinations were done twice; the relative deviation was less than 5%.

\* Samples A–C correspond to MS I type: A, 0–2 cm; B, 2–4 cm; C, 4–6 cm.

† Surface sample from an MS I type stack fragment (comparable to sample A).

‡ Stockwork mineralization of hydrothermally altered rhyolite; analyses of bulk material taken from about 5 cm deeper than the oxidized surface layer.

§ Olive green MS II type sample.

in the more Cu-rich sulphide samples.

The sulphur isotope values  $\delta^{34}\text{S}$  (‰ relative to CDT standard) of 17 pyrite samples from MS I type ores range from +4.3 to +10.7‰, being generally between +6 and +8‰. Values from barite lie within the range +21.9 to +23.6‰. The Jade pyrites have substantially higher  $\delta^{34}\text{S}$  values than other recent sea-floor sulphide deposits at mid-ocean ridges<sup>12,13</sup>, being comparable with Kuroko sulphides, which have  $\delta^{34}\text{S}$  values ranging from +5 to +8‰ (ref. 14). The Jade barites are isotopically similar to other barites, implying a seawater-sulphate origin ( $\delta^{34}\text{S}$  values of modern sea water: +20.3‰).

The Hokuroku district in northern Honshu is the type locality of the Kuroko massive sulphide occurrences, which are found in association with felsic calc-alkaline volcanic rocks<sup>1</sup>. The latter consist of rhyolite and dacite, which intruded to shallow depths below the sea floor or poured out on the sea bottom. They generally underlie stratiform ore bodies, but may also serve as host rocks for stockwork mineralizations. In Kuroko deposits, Au-rich black ores are found stratigraphically above Au-poor, higher-temperature yellow ores and stockwork mineralizations<sup>11</sup>. Our results suggest that the Jade hydrothermal field is analogous, in its nascent state, to the volcanogenic Kuroko-type massive sulphides. □

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## Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna

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**MORE than half of all tropical soils are highly weathered, leached and impoverished, requiring the ecosystem to develop nutrient-conserving mechanisms<sup>1,2</sup>. Nutrient retention and withdrawal mechanisms are most effective in nutrient-poor systems<sup>3,4</sup>. Thus, although dry tropical forests and savanna have the potential capacity to grow at high rates<sup>5,6</sup>, this capacity is strictly limited by climate and nutrients. Our studies on these nutrient-poor ecosystems show that a reciprocal relationship exists between microbial biomass and plant growth rate. This suggests that microbial immobilization may be a main source of nutrients for the plants and may lead to nutrient conservation.**

To study the factors affecting plant growth in nutrient-poor ecosystems, we selected sites in the Vindhyan hill tract (24°20'–25°10' N and 82°30'–83°30' E) of India. The soils at these sites are ultisols, derived from Kaimur sandstones (Dhandraul orthoquartzite). They are reddish brown in colour, sandy loam in texture<sup>6–7</sup>, shallow, leached, and have moderate water-holding capacity (30–50%). The annual rainfall averages 800 mm, of which more than 75% is received during the monsoon season (late June to early September). November to February is a cool dry period (winter) and March to mid-June is hot and dry (summer). Although leaf emergence in most woody species precedes the rainy season, perhaps supported by nutrients withdrawn from senescing leaves, massive biomass accretion coincides with the growth spurt of the herbaceous layer triggered by the first showers of monsoon rain<sup>8</sup>. The herbs start drying up from October, leaving a substantial amount of their roots in the soil.

Every month five samples were collected from the upper 10-cm layers of soil at each site. Ammonium-N and nitrate-N were determined by the phenate and phenol-disulphonic acid methods, respectively, and phosphate-P was determined by the molybdate blue method<sup>9–11</sup>. An *in situ* incubation technique was used to measure nitrification<sup>12</sup>. Microbial C was determined using a modified liquid chloroform fumigation incubation technique, and microbial N and P were determined using fumigation extraction method<sup>13–15</sup>.

The contents of extractable ammonium-N and phosphate-P were low and varied considerably within the annual cycle. In forest soils, nitrate-N varied from 0.4–5.6 µg per g dry soil, and in savanna soils from 0.4–0.8 µg per g. Values for ammonium-N were in the ranges 1.2–4.0 and 2.0–5.0 µg per g for forest and savanna sites, respectively. The contents of phosphate-P extracted using NaHCO<sub>3</sub> varied from 1.4–4.8 µg per g for forest soils and from 2.0–4.0 µg per g for savanna soils; the highest levels of phosphate-P as well as inorganic-N occurred in the summer. There was a marked seasonality in the rate of nitrification which peaked during the rainy season (Fig. 1); all soils showed little or no net nitrification during the dry period. The peak nitrate production rates in these ecosystems were lower than the minimum rates reported for tropical rain forest sites in Brazil, Costa Rica and Panama<sup>16</sup>. Low nutrient pools and low rates of nitrification show that these systems are extremely nutrient poor.

The highest and lowest levels of microbial biomass N occurred in the summer and rainy season, respectively, and the range of

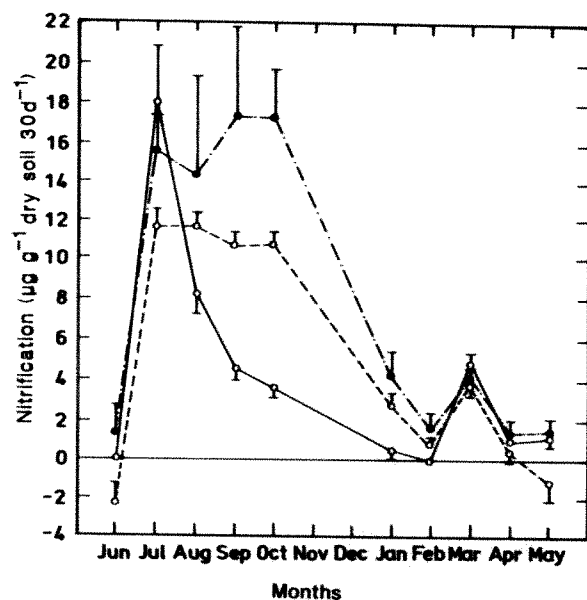


FIG. 1 Nitrification in dry tropical forest and savanna sites. ●—●, Forest; ○—○, grass savanna and ○—○, bamboo savanna (bars, 1 s.e.).



TABLE 1 Microbial biomass C, N and P in dry tropical ecosystems

Season	C	N	P
Forest			
Rainy	487 ± 9.2	51 ± 0.3	20 ± 1.7
Winter	662 ± 14.8	70 ± 1.2	29 ± 3.3
Summer	744 ± 10.3	88 ± 4.1	31 ± 1.6
Savanna			
Rainy	262 ± 7.5	31 ± 0.2	11 ± 1.5
Winter	400 ± 7.5	32 ± 0.7	19 ± 1.6
Summer	520 ± 9.4	46 ± 1.7	23 ± 1.8

Biomass C, N and P contents given as  $\mu\text{g g}^{-1}$  dry soil. Differences due to seasons were significant at  $P < 0.001$  for both the forest and savanna ecosystems.

values measured (31–88  $\mu\text{g per g}$ ) fell within that reported for the Amazonian rain forest sites<sup>17</sup>. Microbial immobilization of  $^{15}\text{N}$  is reported to be greater at sites where the rates of nitrification are low than at sites where they are high<sup>16</sup>. Furthermore, the activity of soil microbes is less sensitive to soil water potential than is water uptake by the plants<sup>18–19</sup>, and it is possible that a substantial amount of water is present at high tension during summer which is unavailable to plants but available to microbes<sup>1</sup>. It seems, therefore, that microbial activity in these systems continues during dry months but that plant growth is drastically reduced, curtailing the demand for nutrients. Mineralization products and nutrients that move upward through capillary action in the dry season are thus immobilized in the microbial biomass.

In the ecosystems studied the microbial biomass and nutrient pools declined as N-mineralization increased, during the period when plant growth was most rapid. This correlation indicates that N immobilized in microbes is an important source of N for plants. This contrasts with the common assumption that steady-state mineralization is responsible for most of the nutrient supply to plants. Accelerated pulsed turnover of microbes in the rainy season may result from grazing by soil animals such as protozoa and nematodes. The populations of protozoa, fungivorous microarthropods and bacteria- and fungi-vorous nematodes expand in this season<sup>20–22</sup>. Grazing of bacteria is necessary to make nutrients available for plant uptake<sup>23–24</sup>. Mineral N is released mainly from dead microbial cells, microbial materials and microbial metabolites<sup>25</sup>. Fresh plant material has to be partly humidified before mineral N might be released<sup>26</sup>. The turnover of N from dead microbial cells is about five times that from native soil organic N<sup>27–28</sup>. In low-N soils, microbial biomass is as effective as nitrate-N in supplying N to wheat<sup>29</sup>. Vitousek and Matson<sup>30</sup> have concluded that microbial biomass, if conserved during forest management, retains N in harvested loblolly pine plantations.

We conclude that microbial biomass in these nutrient-poor systems acts as a sink and a source of nutrients. The principal function of the microbial biomass is thus to accumulate and conserve nutrients in a biologically active form during the dry period (high biomass, low turnover), when the activity of the plants is low and they are not able to extract nutrients effectively from soil, and then to release them rapidly during the monsoon period (low biomass, high turnover) to initiate plant growth. □

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## Temporally distinct pre- and post-synaptic mechanisms maintain long-term potentiation

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LONG-TERM potentiation (LTP) in the hippocampus is widely studied as the mechanisms involved in its induction and maintenance are believed to underlie fundamental properties of learning and memory in vertebrates<sup>1</sup>. Most synapses that exhibit LTP use an excitatory amino-acid neurotransmitter that acts on two types of receptor, the N-methyl-D-aspartate (NMDA) and quisqualate receptors<sup>2</sup>. The quisqualate receptor mediates the fast synaptic response evoked by low-frequency stimulation<sup>3,4</sup>, whereas the NMDA receptor system is activated transiently by tetanic stimulation, leading to the induction of LTP<sup>3,5–7</sup>. The events responsible for maintaining LTP once it is established are not known. We now demonstrate that the sensitivity of CA1 neurons in hippocampal slices to ionophoretically-applied quisqualate receptor ligands slowly increases following the induction of LTP. This provides direct evidence for a functional post-synaptic change and suggests that pre-synaptic mechanisms also contribute, but in a temporally distinct manner, to the maintenance of LTP.

The excitatory post-synaptic potential (e.p.s.p.) evoked in CA1 neurons by low frequency stimulation of the Schaffer collateral-commissural pathway (see Fig. 1a) is depressed by the quisqualate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)<sup>4,8–10</sup>. In physiological medium, NMDA antagonists do not affect this e.p.s.p. either before or after the induction of LTP<sup>3</sup>; it is therefore assumed that the potentiated e.p.s.p. is mediated by quisqualate receptors. To test this directly, we compared the sensitivity to CNQX of potentiated and unpotentiated inputs onto the same population of CA1 neurons. As illustrated in Fig. 1, 10  $\mu\text{M}$  CNQX, a concentration that depresses responses of CA1 neurons to quisqualate and AMPA

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but not NMDA<sup>4</sup>, depressed both inputs in parallel ( $n = 6$ ). Thus, the receptors that mediate the potentiated response are probably of the quisqualate receptor subtype. This result means that if LTP is maintained by post-synaptic mechanisms there should be an associated increase in the sensitivity of neurons to appropriately administered quisqualate receptor ligands.

To test for this, intracellular recordings were obtained from CA1 pyramidal neurons (Fig. 2). In five cells, tetanic stimulation elicited immediate and stable LTP; the respective mean ( $\pm$ s.e.m.) amplitudes of the e.p.s.p., measured from averages of 10 successive responses, 0–5 min before and 25–30 min after the induction of LTP were  $5.3 \pm 0.8$  and  $8.5 \pm 1.3$  mV (mean increase,  $53 \pm 12\%$ ). The generation of LTP was not associated with any significant change in either the resting membrane potential or resting input resistance; the values at the corresponding times were  $-69 \pm 2$  and  $-70 \pm 2$  mV, and  $29 \pm 2$  and  $31 \pm 3$  M $\Omega$ , respectively. In these cells, quisqualate elicited depolarizations of  $5.7 \pm 0.7$  mV, measured from averages of 10 successive responses 0–5 min before the induction of LTP. By contrast with the synaptic response, quisqualate-induced depolarizations were not immediately affected by tetanic stimulation. For example, the mean depolarization 5–10 min after the induction of LTP was still  $5.7 \pm 0.7$  mV. Thereafter, however, there was a tendency for the quisqualate-induced responses to increase in size. In four cells there was an increase ( $21 \pm 6\%$ ) by 25–30 min post-tetanus and in the fifth cell the response size showed a comparable increase by 60 min. By contrast, in three cells tetanic stimulation resulted in neither the generation of LTP nor an increase in sensitivity to quisqualate. Thus, in only those cells where stable LTP had been induced was there a delayed increase in sensitivity to quisqualate.

To quantify the effect further, stable extracellular d.c. potentials<sup>11</sup> were obtained in response to ionophoretic administration of the selective quisqualate receptor agonist AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) at 15-min intervals for 1 h before and 3 h following tetanic stimulation (Fig. 3). In control medium this elicited LTP in 9 out of 11 slices;

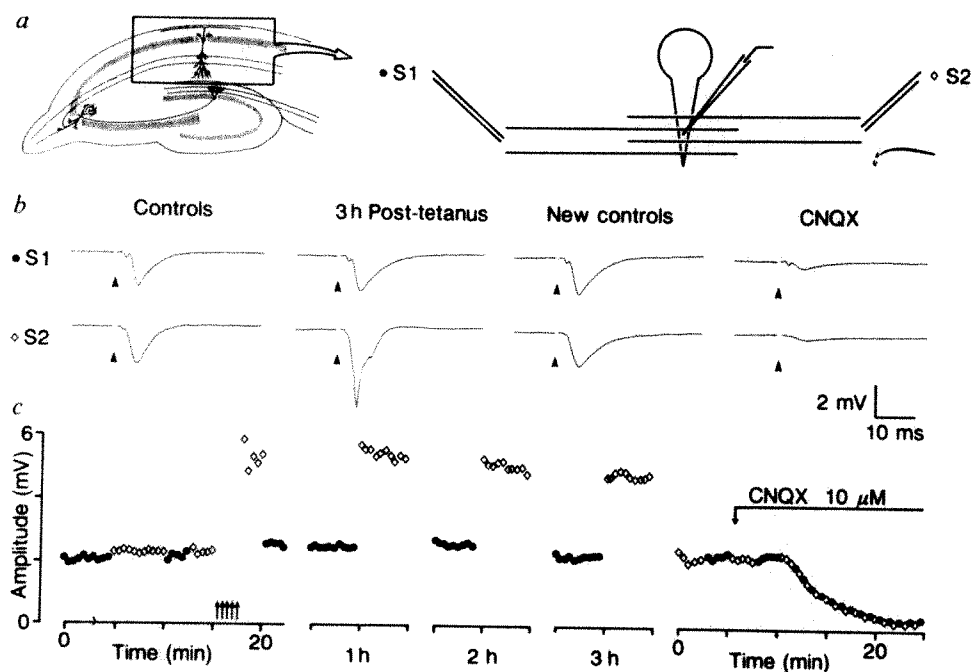
the mean change in the field e.p.s.p. amplitude (and slope) 2 h after the tetanus for all 11 slices was  $+47 \pm 21\%$  ( $+82 \pm 34\%$ ) of control. Usually the sensitivity of the cells to the first post-tetanus AMPA application was unaltered. In most cases, however, the sensitivity to AMPA then gradually increased, reaching a maximum after about 2 h and persisting throughout the 3-h period following the tetanus. The mean change for all 11 slices at 2 h was  $+62 \pm 25\%$  ( $P < 0.05$ ; paired  $t$ -test).

If the change in AMPA sensitivity is the result of the generation of LTP, it should be blocked by treatments that prevent the induction of LTP, such as adding an NMDA antagonist<sup>3</sup>. In the presence of  $50 \mu\text{M}$  D-2-amino-5-phosphonovalerate (APV) there was neither the production of LTP (mean change in field e.p.s.p. 2 h after tetanus was  $-9 \pm 9\%$  ( $-2 \pm 8\%$ ) of control), nor any consistent change in the sensitivity of CA1 neurons to AMPA (Fig. 3). The mean change in the amplitude of the AMPA responses 2 h post-tetanus was  $-7 \pm 12\%$  ( $P > 0.05$ ; paired  $t$ -test). In three slices, tetanic stimulation was delivered first in the presence of APV and, 3 h later, after washout of APV; invariably APV reversibly prevented both the induction of LTP and the sensitivity change to AMPA (Fig. 3).

To examine whether the potentiated AMPA response involved activation of local synaptic circuitry, we tested the effects of a  $\text{Ca}^{2+}$ -channel antagonist on responses 2 h after tetanization. In these slices, synaptic and AMPA-induced responses had increased 2 h post-tetanus by  $33 \pm 7\%$  ( $62 \pm 15\%$ ) and  $43 \pm 8\%$  ( $n = 4$ ), respectively. Cadmium ( $50$ – $100 \mu\text{M}$ ) reduced the amplitude of the synaptic response by  $56$ – $70\%$  but had no effect on the amplitude of the AMPA-evoked depolarizations.

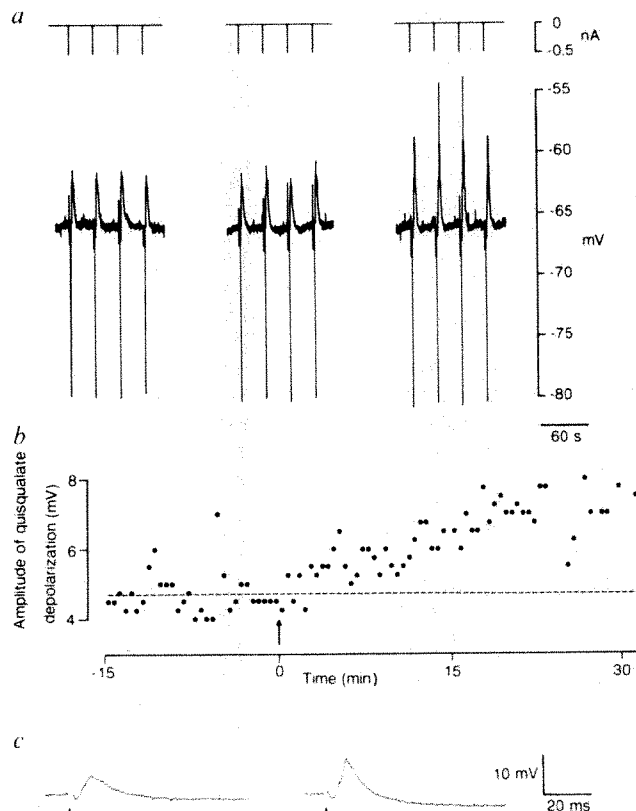
Whether LTP is maintained by pre-synaptic<sup>12–14</sup> or post-synaptic<sup>15–17</sup> mechanisms is controversial. Our data suggest that in the Schaffer collateral-commissural pathway both mechanisms occur but with different time courses. Previous studies have failed to detect an increase in sensitivity to ionophoretically administered agonists with LTP<sup>18–20</sup>. There are two explanations for this. First, the sensitivity to exogenously applied agonists was followed only for short periods and, as we show, sensitivity

FIG. 1 CNQX blocks in parallel potentiated and unpotentiated field e.p.s.p.s. a, Diagrammatic representation of the recording arrangement for this series of experiments; an extracellular recording electrode was placed in stratum radiatum of area CA1 and two stimulating electrodes (S1 and S2) were positioned  $\sim 1$  mm on either side. A large separation was used to minimize the current to which both electrodes stimulated the same fibres. LTP was induced by stimulating one input using five trains delivered at 30-s intervals. Each train comprised 100-Hz stimulation for 1 s at double test intensity. After the induction of LTP (15, 30 or 180 min) the amplitude of the two inputs was matched by increasing the stimulus intensity of the control input or by decreasing that of the potentiated input and CNQX was then applied. b, An example from one slice of field e.p.s.p.s. evoked by the two stimulating electrodes showing from left to right, matched control responses (2.8 and 3.0 V), responses after tetanization of S2, new matched controls after S2 intensity had been reduced to 2.1 V, and finally the effect of CNQX ( $10 \mu\text{M}$ ) on the two responses. In these and subsequent figures synaptic records are averages of 3–10 consecutive responses and the position of the blanked stimulus artefacts are indicated by arrowheads. The time of tetanization is shown by arrows. c, Amplitude of the field e.p.s.p.s. plotted throughout the experiment illustrated in b showing the parallel reduction by CNQX of the potentiated and the unpotentiated response.



METHODS. Rat hippocampal slices ( $400\text{-}\mu\text{m}$  thick) were prepared and maintained in an interface type chamber at  $30$ – $32^\circ\text{C}$ , using standard techniques<sup>3</sup>. They were perfused with medium containing: NaCl,  $124$  mM; KCl,  $3$  mM;  $\text{NaHCO}_3$ ,  $26$  mM;  $\text{CaCl}_2$ ,  $2$  mM;  $\text{MgSO}_4$ ,  $1$  mM; D-glucose,  $10$  mM;  $\text{NaH}_2\text{PO}_4$ ,  $1.25$  mM (omitted for cadmium experiments), bubbled with a  $95\%$   $\text{O}_2/5\%$   $\text{CO}_2$  mixture.

changes are usually slow to develop. Second, L-glutamate was used which, unlike AMPA and quisqualate in hippocampal slices<sup>4</sup>, exerts only part of its action through CNQX-sensitive receptors<sup>21</sup>. Our results favour a pre-synaptic component to the early maintenance of LTP in the Schaffer collateral-commissural pathway because LTP was produced within 30 s, yet sensitivity changes were not usually detected for at least 15 min and took ~2 h to reach a maximum.



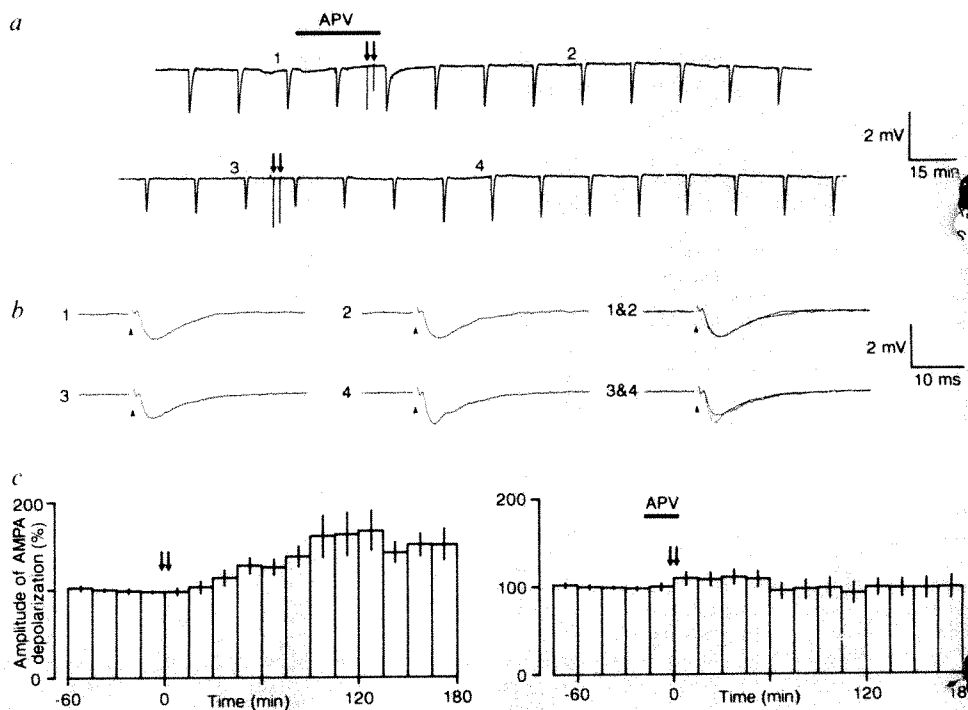
An intriguing feature of the post-synaptic change is its slow development. Interestingly, in the presence of protein kinase C (PKC) inhibitors, LTP lasts for only 1–2 h (refs 22–24); a time similar to that required for maximal potentiation of the AMPA-induced responses. Moreover, injection of purified PKC into the post-synaptic neuron induces an LTP-like change in synaptic transmission<sup>25</sup>. It is possible that PKC contributes to the initiation of this slowly developing, postsynaptically-located com-

FIG. 2 LTP is associated with a delayed increase in sensitivity of single CA1 neurons to quisqualate. *a*, Current (top) and voltage (bottom) records from an intracellularly recorded CA1 pyramidal neuron. Depolarizations are in response to ionophoretic ejection of quisqualate (130 nA, 2 s) into stratum radiatum. These are preceded by hyperpolarizations in response to constant current pulses (0.5 nA, 400 ms), used to monitor resting input resistance. Panels illustrate from left to right, control records, sections taken immediately after, and sections taken 30 min after the tetanus (100 Hz, 1 s, test intensity). Two of the last four quisqualate-induced responses depolarize the cell sufficiently to fire action potentials. *b*, Plot showing the amplitude of quisqualate-induced depolarizations for 15 min before and 30 min following the tetanus (arrow). Thereafter (for a further 45 min) quisqualate-induced depolarizations excited the cell sufficiently to elicit firing. *c*, Synaptic responses taken immediately before and 30 min after the tetanus.

**METHODS.** Intracellular recordings were obtained from the cell-body layer using electrodes containing 3 M potassium acetate and the Schaffer collateral-commissural pathway was stimulated at 30 s intervals by a single electrode placed in stratum radiatum. An ionophoretic pipette was positioned in stratum radiatum at the level of the stimulated fibres, adjacent to the recording site. Quisqualate (20 mM in 150 mM NaCl) was applied ionophoretically (46–163 nA, 1–2 s) 5 s after each synaptic response was elicited. The position of the ionophoretic pipette was adjusted to achieve a compromise between short latency responses (indicating that the pipette was close to a dendritic branch of the recorded cell) and the maintenance of stable recordings. Only those cells where passive membrane properties and both synaptic and agonist-induced responses remained stable for 15 min before tetanic stimulation (100 Hz, 1 s, test intensity) and where healthy impalements could be maintained for at least a further 30 min were included in the analysis.

FIG. 3 The delayed increase in sensitivity is dependent on the synaptic activation of NMDA receptors. *a*, d.c. potentials evoked in the stratum radiatum by ionophoretic ejection of AMPA (87 nA, 20 s) every 15 min. In the upper panel APV (50  $\mu$ M) was present during the tetani and there was minimal change in the size of the AMPA-induced responses. In the lower panel a second pair of tetani delivered 3 h later in the absence of APV induced a delayed increase in the amplitude of the AMPA-induced responses. The two sections of record are continuous. *b*, Field e.p.s.ps recorded at times indicated, showing synaptic responses before and 1 h after the tetani. *c*, Pooled data showing effect of tetanization on amplitude of AMPA responses (normalized to mean of control responses) in the absence ( $n=11$ ) and presence ( $n=8$ ) of APV (50  $\mu$ M). Error bars show  $\pm 1$  s.e.m.

**METHODS.** The experimental arrangement was similar to that shown in Fig. 1, except that the two stimulating electrodes were placed closer together to maximize the number of tetanized synapses in the vicinity of the recording electrode. Each pathway was tetanized once (100 Hz, 1 s, double test intensity). AMPA (20 mM in 150 mM NaCl) was applied ionophoretically



(40–155 nA, 10–25 s, at 15 min intervals) from a pipette close to the recording electrode.



ponent of LTP. The early presynaptic component presumably involves PKC-independent mechanisms. One possibility is that NMDA receptor activation releases arachidonic acid metabolites<sup>26</sup> from the post-synaptic cell and that these act as retrograde messengers which result in the enhanced neurotransmitter release<sup>27,28</sup>.

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## Presentation of antigen, foreign major histocompatibility complex proteins and self by thymus cortical epithelium

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IN mouse and man most peripheral T cells bear clonally variable receptors made up of  $\alpha$ - and  $\beta$ -chains<sup>1</sup> which bind ligands on target cells consisting of peptide fragments of foreign antigens, complexed with cell surface proteins encoded by the major histocompatibility complex (MHC) of the individual<sup>2–4</sup>. In the thymus, developing T cells are selected to mature only if their receptors will be able to participate in self-MHC plus antigen recognition in the periphery<sup>5–8</sup>. This positive selection occurs in the presence of self-MHC, but in the apparent absence of antigen, leading to the paradoxical conclusion that developing thymocytes must be positively selected by engagement of their receptors and self-MHC alone, although thymocytes that react too well with self-MHC are eliminated<sup>9</sup>. To account for this, it has been suggested that MHC molecules in the thymus are not identical to those found elsewhere<sup>1</sup>. To test this and other hypotheses, we have examined the ability of the presumed selecting cells, those of the thymus cortical epithelium<sup>10,11</sup>, to present various MHC complexes to T cells. Our results indicate that MHC molecules on thymus epithelium are not always the same as those found elsewhere.

Thymus cortical epithelial cells were isolated as nurse cells<sup>12</sup> from BALB/c (H-2<sup>d</sup>) mice and tested for their ability to present antigen plus I-A<sup>d</sup>, or I-E<sup>d</sup> to T-cell hybridomas. T-cell-depleted BALB/c spleen cells were used as controls. As shown in Fig. 1a and b, a T-cell hybridoma specific for I-E<sup>d</sup> as an alloantigen, CG4, responded equally well to the nurse cell and spleen cell preparations in the dose ranges tested. Similar results were obtained using a T-cell hybridoma from BALB/c mice, specific for a peptide of chicken ovalbumin (cOVA)/I-A<sup>d</sup>, 3DO-8.2 (Fig. 1c, d). Kyewski *et al.*<sup>13</sup> have previously shown that thymus epithelial cells cannot present antigen to T-cell clones. The discrepancy between their results and those shown here may be due to our use of T-cell hybridomas, cells which, unlike the normal T cells used by Kyewski *et al.*, require only  $\alpha/\beta$ -receptor engagement and no secondary signals to respond.

Our results show that some cells in the thymus epithelium preparation can present allogeneic MHC or antigen plus self-MHC to T-cell hybridomas. Although the active cells could be contaminant macrophages or dendritic cells, it seems that the epithelial cells themselves contribute to the presentation: not only did the nurse-cell preparations contain no or few rosette-forming dendritic cells or macrophages, but their antigen-presenting activity did not fall off upon selectively enriching for nurse cells (data not shown). Also, as shown below, the nurse cell preparations were selectively deficient in their ability to present antigen-MHC complexes which are expressed on B cells and macrophages *in vivo*.

Recently it has been shown that almost all T-cell receptors that include V $\beta$ 17a impose reactivity for IE plus a B-cell-derived product on T cells which bear them<sup>14,15</sup>. Despite this, V $\beta$ 17a<sup>+</sup> cells seem to react with I-E on macrophages isolated from mice, perhaps because of transfer of the B-cell-derived product from one cell to another *in vivo*. Indirect evidence, however, suggested that the V $\beta$ 17a ligand might not exist on selecting cells in the thymus, as V $\beta$ 17a<sup>+</sup> cells are not 'overselected' in thymuses which express I-E on thymus epithelium, but elsewhere<sup>16</sup>. To test this, three V $\beta$ 17a<sup>+</sup> T-cell hybridomas were challenged with I-E<sup>d</sup> on BALB/c nurse or spleen cells. The hybridomas responded poorly to nurse cells, and much better to spleen cells (Fig. 1e, f). This indicates that V $\beta$ 17a<sup>+</sup> T cells do not react well, if at all, with IE on thymus nurse cells, as the small responses seen against our epithelial cell preparations may have been against contaminating cells. Preliminary experiments show that nurse cells from transgenic mice, expressing I-E on only thymus epithelial cells, do stimulate weak responses from some V $\beta$ 17a<sup>+</sup> hybrids, indicating that the V $\beta$ 17a ligand might be present on thymus epithelium, albeit at low levels.

We also tested the ability of T-cell hybrids to react with syngeneic thymus epithelium in the absence of antigen. A BALB/c-derived T-cell hybridoma specific for cOVA/I-A<sup>d</sup>, 3DO-54.8, reacted with the BALB/c nurse cell preparation in the absence of antigen (Fig. 1g, h). This T cell did not react with spleen cells in the absence of cOVA, even when the spleen cells had been subjected to the same enzyme treatments as the nurse cells, nor did it react with BALB/c-derived, I-A<sup>d</sup>-bearing macrophages or B-cell lymphomas. The failure of 3DO-54.8 to react with spleen cells was not due to a splenic inhibitory activity, as mixtures of splenic and nurse cells stimulated this and another hybrid as well as the nurse cell preparations alone (data not shown).

Several independent T-cell hybridomas were tested for their ability to react with syngeneic nurse cells (Fig. 2). The BALB.B (H-2<sup>b</sup>)-derived hybridomas, BO-97.10 and 2BO-43 responded quite well to H-2<sup>b</sup>-bearing nurse cell; BO-97.10 responded only slightly to spleen cells from the same animals. These hybridomas did not react significantly with H-2<sup>d</sup> nurse or spleen cells. A third H-2<sup>b</sup>-derived hybridoma, 2BO-33, did not react with any preparation, and two H-2<sup>d</sup>-derived hybridomas, 22DO-14 and 3DO-54.8, reacted with H-2<sup>d</sup> nurse but not H-2<sup>d</sup> spleen cells. The hybridoma 3DO-54.8 failed to react with the H-2<sup>b</sup> cell

preparations, and 22DO-14 reacted only slightly with both nurse and spleen cell preparations from the H-2<sup>b</sup> mice. Of 35 T-cell hybridomas screened, at least 54% react to some degree with nurse cells from syngeneic mice. By contrast, 26% respond to syngeneic spleen cells, or allogeneic nurse or spleen cells. Usually the reactivities to syngeneic nurse cells are small by comparison with responses to specific antigen plus MHC, or anti-receptor antibody, by the same hybrids. We have been unable to enhance these reactivities by increasing the incubation times of the nurse cells with the T cells, or by adding gamma interferon or indomethacin.

Many of the hybridomas that responded to syngeneic nurse cells were produced by fusion of normal T cells to a derivative of the thymoma, BW 5147 (ref. 17), which does not express functional T-cell receptor  $\alpha$ - and  $\beta$ -chain messenger RNA. The only T-cell receptors expressed on these cells which could be involved in reaction with the syngeneic thymus epithelial cells were therefore those derived from their normal T-cell parents. The hybridoma 3DO-54.8, however, was produced by fusion of a normal T cell to BW5147, which itself contains functional  $\alpha$ - and  $\beta$ -chain mRNA; it may thus express up to four different receptors on its surface, by combination of the normal T-cell and BW receptor polypeptides.

To show that the BALB/c-selected cOVA/I-A<sup>d</sup> receptor on 3DO-54.8 was responsible for the reactivity of this hybrid with syngeneic nurse cells, we tested it and several of its derivatives under various conditions for their ability to respond to BALB/c nurse cells. As before, 3DO-54.8 responded to the nurse cells; this response was inhibited by antibodies to the restricting MHC molecule for 3DO-54.8, I-A<sup>d</sup>. A subclone of the hybridoma, 3DO-54.8.21, in which the  $\beta$ -chain gene inherited from its nor-

mal T-cell parent is absent, did not respond to syngeneic nurse cells, nor did a subclone of 3DO-54.8, 3DO-54.8.43, which no longer expresses CD4. It seems therefore that reaction of 3DO-54.8 with BALB/c nurse cells involves I-A<sup>d</sup> on the nurse cells, CD4 and, probably, the BALB/c T-cell-derived receptor on 3DO-54.8.

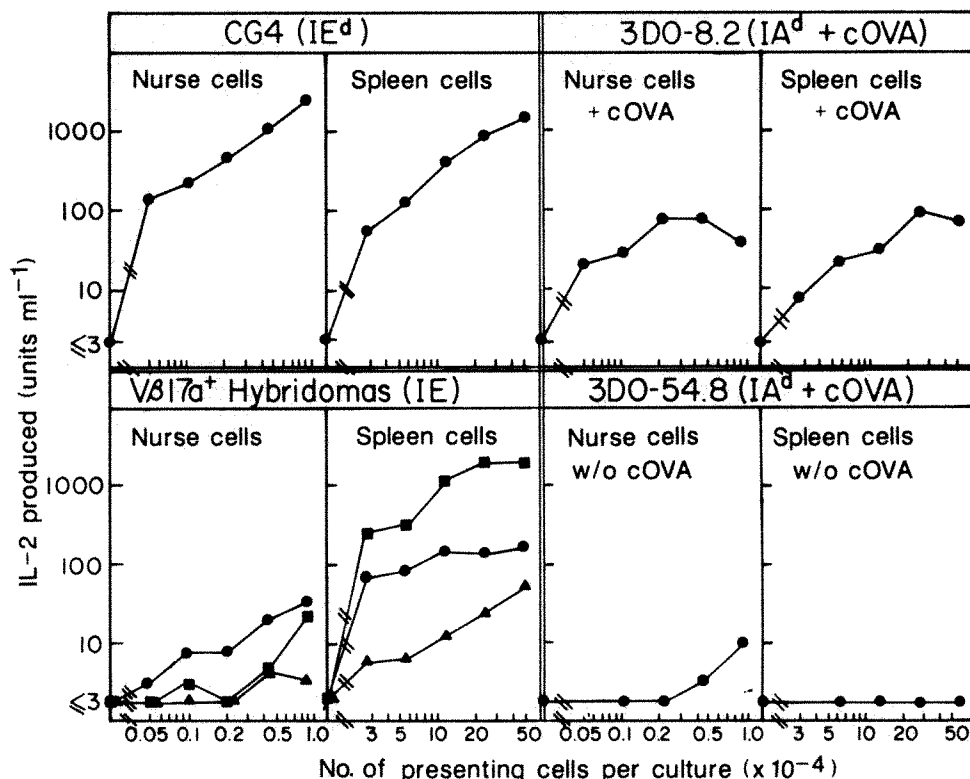
Overall, these results indicate that MHC on thymus cortical epithelial cells is not always seen by T cells in the same way as MHC expressed on other cell types. Although nurse cells present antigen plus self-MHC, and in some cases allogeneic MHC, very effectively to some T cells, they are defective in their ability to present alloantigen to other cells: in the present case, I-E to V $\beta$ 17a<sup>+</sup> T cells. As the ligand for V $\beta$ 17a<sup>+</sup> receptors is thought to be a complex of I-E and a B-cell-derived ligand<sup>15</sup>, it is not surprising that thymus cortical epithelial cells, which probably have limited access to B-cell products, do not present it effectively.

More interestingly, MHC on thymus cortical epithelial cells, but not on other cells, can in some cases stimulate hybridomas made from syngeneic T cells, a reaction which may reflect the process of positive selection. These syngeneic responses do not occur with all T cells and are very small, suggesting that the combination of positive selection and tolerance allows only those cells with very weak reactivity for self-MHC to develop into mature T cells, a possibility suggested previously by the work of Ron *et al.*<sup>10,11</sup>. Alternatively, the weak, variable results may reflect the fact that our tests *in vitro* are poor models of the situation *in vivo*, where positive selection probably involves very intimate contact between thymus epithelium and CD4<sup>+</sup>, CD8<sup>+</sup> thymocytes<sup>18</sup>. In either case, the response of T cells to syngeneic thymus epithelial cells, but not to spleen cells, may

FIG. 1 Presentation of antigen, allogeneic MHC and self by thymus cortical epithelial cells.

**METHODS.** Thymuses were removed from 40 6-week-old BALB/c mice, and nurse cells prepared as previously described<sup>12</sup>, using a combination of mechanical disruption, digestion with collagenase (0.1 U ml<sup>-1</sup>, from *Achromobacter iophagus*, Boehringer), dispase (0.8 U ml<sup>-1</sup>, from *Bacillus polymixa*, Boehringer) and bovine pancreas DNase I (0.05 mg ml<sup>-1</sup>), and gravity sedimentation on fetal bovine serum step gradients in the presence of 50 mM EDTA. In the experiment shown,  $2.4 \times 10^5$  nurse cells were obtained, contaminated with an equal number of free thymocytes. Spleen cells were treated with anti-T-cell serum and complement to remove T cells before use as antigen-presenting cells. Nurse or spleen cells were titrated into 250  $\mu$ l microcultures containing  $10^5$  T hybridoma cells and cOVA, where required, at 1 mg ml<sup>-1</sup>. After 24 h, supernatants from these cultures were assayed for interleukin-2 (IL-2) content using HT-2 cells and MTT as an indicator of cell survival<sup>19,20</sup>. This method allows  $<2$  U ml<sup>-1</sup> of IL-2 to be measured accurately. Control cultures, set up with nurse or spleen cells alone,

or with a T-cell hybridoma with no reactivity for BALB/c targets, contained no detectable IL-2. The results shown are those from a single experiment, representative of five similar experiments. Pairs of panels show IL-2 titres with the indicated T-cell hybridomas assayed on nurse or spleen cells. CG4 was produced from a fusion of C57BL/6 anti-BALB/c T cells to BW5147, and is specific for I-E<sup>d</sup>. 3DO-8.2 was produced from a fusion of BALB/c (H-2<sup>d</sup>), cOVA-primed T cells to BW5147. In this experiment 3DO-8.2 showed



no response to BALB/c nurse or spleen cells in the absence of cOVA. 36-19-21 (■), 2Q23-34.7.9 (●) and Q023-26.9 (△) were three V $\beta$ 17a<sup>+</sup> T-cell hybridomas, produced by fusion of SWR T cells to an  $\alpha$ -derivative of BW147 (ref. 17). The 3DO-54.8 was produced from a fusion of cOVA-primed, BALB/c T cells to BW5147. The hybridoma is specific for cOVA/I-A<sup>d</sup>. In this experiment it gave 119 U/ml<sup>-1</sup> IL-2 when challenged with cOVA and BALB/c spleen cells.

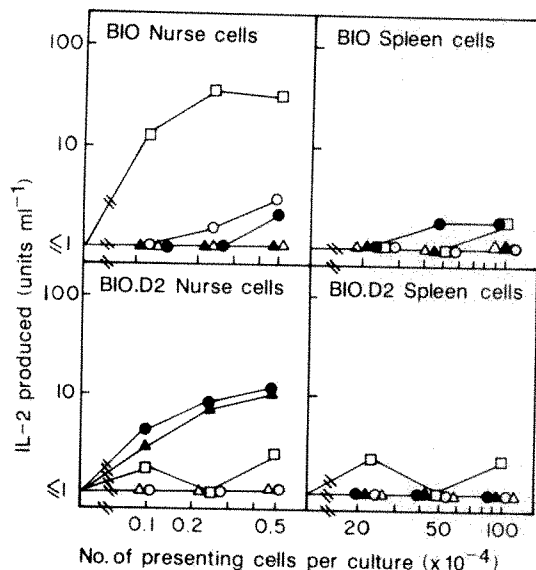


FIG. 2 Some T-cell hybridomas react specifically with syngeneic thymus cortical epithelial cells. Nurse cells were prepared from 19 B10 (H-2<sup>b</sup>) or B10.D2 (H-2<sup>d</sup>) 6-8-week-old mice as described in the legend to Fig. 1. These, and anti-T- and complement-treated spleen cells from the same animals, were tested for their ability to stimulate T-cell hybridomas bearing T-cell receptors from H-2<sup>b</sup> (open symbols) or H-2<sup>d</sup> (closed symbols) mice. Origins of the T-cell hybridomas: BO-97.10 (□) was produced from the fusion of cOVA-primed, C57BL/10 (B10, H-2<sup>b</sup>) T cells to BW5147. 2B0-33 (Δ) and 2B0-43 (○) were also produced from cOVA-primed, B10 cells, but these were fused to an  $\alpha^-$ ,  $\beta^-$ -derivative of BW5147 (ref. 17). 3D0-54.8 (●) was produced as described in the Fig. 1 legend, 22D0-43 (▲) was produced by fusion of cOVA-primed B10.D2 (H-2<sup>d</sup>) T cells to the  $\alpha^-$ ,  $\beta^-$ -derivative of BW5147.

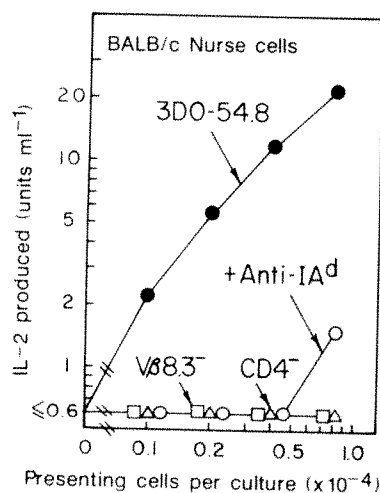


FIG. 3 The normal T-cell-derived receptor and CD4 are required for recognition of restricting I-A on syngeneic thymus cortical epithelial cells by a T-cell hybridoma.

**METHODS.** Nurse cells were prepared from 40 5-week-old BALB/c mice as described in Fig. 1 legend. These, and anti-T- and complement-treated spleen cells, were assayed for their ability to stimulate the BALB/c-derived T-cell hybridoma, 3D0-54.8 (●), and its derivatives, as described in Fig. 1. 3D0-54.8.21 (□) and 3D0-54.8.43 (Δ) were produced by subcloning after prolonged passage of 3D0-54.8 in culture. Southern blots showed that 3D0-54.8.21, which no longer reacts with cOVA/I-A<sup>d</sup>, has lost the only normal T-cell-derived, rearranged  $\beta$ -chain gene characteristic of 3D0-54.8. 3D0-54.8.43, unlike its parent, bears no surface CD4, and reacts very weakly with cOVA/I-A<sup>d</sup>. The anti-I-A<sup>d</sup> monoclonal antibody, MK-D6 was used as a 20% concentration of culture supernatant from the antibody-producing cell line (○).

occur because of quantitative or qualitative differences in MHC expression between thymus epithelial and peripheral cells. We find that thymus cortical epithelial cells do not express a higher density of MHC proteins than cells such as some B-cell lymphomas. Of these two explanations, we therefore prefer the latter; but, of course, the two theories are not mutually exclusive, and both may apply for different T cells. □

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## Severe immunodeficiency disease induced by a defective murine leukaemia virus

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**DIFFERENT** classes of retroviruses have been shown to induce immunodeficiency diseases in various animal species<sup>1</sup>. These animal models may provide an insight into our understanding of AIDS<sup>1-3</sup> but, with the exception of one strain of feline leukaemia virus<sup>4</sup>, the determinants of pathogenicity have not yet been mapped to these viral genomes. The immunodeficiency-inducing feline leukaemia virus is replication-defective<sup>4</sup>, harbouring the determinant of pathogenicity within its *env* sequences<sup>5</sup>. We have studied the Duplan strain of murine leukaemia virus<sup>6</sup> which induces, in C57BL/6 mice<sup>7-11</sup>, a severe immunodeficiency disease with striking similarities to human AIDS<sup>2,3,8,9</sup>. We have identified the aetiological agent of this murine immunodeficiency disease as another defective retrovirus, with a genome of 4.8 kilobases. Molecular cloning and sequencing of this DNA showed that the *pol* and *env* genes have been deleted, but that the complete *gag* region has been conserved and has a novel sequence encoding the p12 protein. As with the feline leukaemia virus<sup>4,5</sup>, these results provide evidence for the role of defective retroviruses in inducing immunodeficiency and facilitate the study of the mechanisms underlying the pathogenesis of retrovirus-induced immunodeficiency syndromes, including AIDS.

A crude extract containing a mixture of different strains of Duplan murine leukaemia viruses (MuLVs), prepared from a lymph node of a diseased mouse, was used to infect SC-1 cells *in vitro*. Filtered supernatant (SC-1/Dup MuLV) from the

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infected cells induced typical disease within 8 to 12 weeks of inoculation of young adult C57BL/6 mice (Table 1).

To analyse individual viruses present in this crude extract and to identify the retrovirus strain involved in the disease, we first analysed unintegrated viral DNA extracted by the Hirt procedure from SC-1 cells acutely infected with SC-1/Dup MuLV. Using a representative retroviral probe, we detected two

major species of linear viral DNA of 8.8 and 4.8 kilobases (kb), corresponding to a typical full-length non-defective MuLV genome, and to a defective retroviral genome, respectively (Fig. 1a, lane 1). This defective viral genome seemed to be as abundant as the non-defective species in this Hirt supernatant. The viral DNA species in the Hirt supernatant were separated into supercoiled and linear or open-circular viral DNA. The defective

TABLE 1 Biological characteristics of cloned Du5H defective MuLV

Virus injected	Mice with lymphadenopathy and splenomegaly	0	[ <sup>3</sup> H]thymidine incorporation (c.p.m. × 10 <sup>-3</sup> )			Serum IgM (mg ml <sup>-1</sup> )
			Con A (μg ml <sup>-1</sup> )	2.5	5.0	
No virus	0 (8)	2.8 ± 0.4	84 ± 17	148 ± 18	118 ± 22	0.25 ± 0.03
G <sub>6</sub> T <sub>2</sub>	0 (8)	6.4 ± 3.0	72 ± 35	152 ± 36	152 ± 26	0.30 ± 0.01
Du5H (G <sub>6</sub> T <sub>2</sub> )	8 (8)	1.4 ± 0.2	1.4 ± 0.3	1.6 ± 0.3	2.0 ± 0.5	1.60 ± 0.40
Duplan extract	8 (8)	1.8 ± 0.5	2.0 ± 0.5	2.1 ± 0.5	2.3 ± 0.4	1.65 ± 0.15

pDu5HNeo DNA was transfected into SIMR cells by the calcium phosphate method<sup>18</sup> and neomycin-resistant colonies were grown in the presence of Geneticin (G418) (200 μg ml<sup>-1</sup>). Twenty-two colonies were picked and rescued with non-pathogenic G<sub>6</sub>T<sub>2</sub> RadLV (ref. 12). To select for the best producer, viruses were collected from the supernatant of each clone, recovered by centrifugation (50 Ti, 35,000 r.p.m. for 30 min) and their RNA extracted by the urea-SDS method<sup>19</sup>. Viral RNA was hybridized with the <sup>32</sup>P-labelled Du5H-specific D30 probe. The best producer was selected to produce stocks. As estimated by hybridization, these stocks contained an approximately three-fold higher concentration of helper viruses than Du5H viruses and the syncytium XC titre<sup>20</sup> of the helper MuLV was 5 × 10<sup>3</sup> plaque-forming units per ml. Young adult (30–40-day-old) C57BL/6 mice were injected intraperitoneally with 0.5 ml twice, one week apart, with medium alone, G<sub>6</sub>T<sub>2</sub> RadLV alone, Du5H rescued with G<sub>6</sub>T<sub>2</sub> RadLV, or with the crude extract of Duplan RadLV. Mice were killed 12–17 weeks after injection when they showed advanced signs of disease (lymphadenopathy). Peripheral lymph-node size varied from 0.4 to 1.5 cm. Spleen cells were suspended into RPMI 1640 medium (Gibco) with 10% fetal calf serum, penicillin G (Sigma) 100 units ml<sup>-1</sup>, streptomycin (Sigma) 100 mg ml<sup>-1</sup> and 10<sup>-5</sup> M β-mercaptoethanol. Aliquots containing 2 × 10<sup>5</sup> cells per 200 μl were distributed in wells of microtest plates (Costar) and incubated at 37 °C for 48 h in humidified atmosphere containing 5% CO<sub>2</sub> with concanavalin A (Con A, Sigma) at 2.5, 5.0 or 10 μg ml<sup>-1</sup>. Cells were incubated for 4 h with 1 μCi of [<sup>3</sup>H]thymidine (42 Ci mmol<sup>-1</sup>) (Amersham) and collected in an automated sample harvester (PHD, Cambridge Technology). Incorporated precursor was counted in a scintillation spectrometer (1215 Rack Beta II, Wallac). The standard error was calculated from an average of 3–5 mice. The concentration of IgM in the sera was measured by a competitive enzyme-linked immunosorbent assay. Polystyrene microtitre plates (Nunc) were coated with affinity-purified mouse IgM (Sigma). Serial dilutions of serum from each mouse were assayed for its ability to inhibit the binding of alkaline phosphatase-conjugated, affinity-purified rabbit anti-mouse IgM (ICN). p-nitrophenyl disodium phosphate (Sigma; 1 mg ml<sup>-1</sup>) was used as a substrate in 1M diethanolamine-HCl (pH 9.8) and 50 mM MgCl<sub>2</sub> and the colour read in the Biotek Model 310 Autoreader at 405 nm. Standard inhibition curves were compared with competitive binding with unconjugated mouse IgM. The standard error was calculated from an average of 3–5 mice.

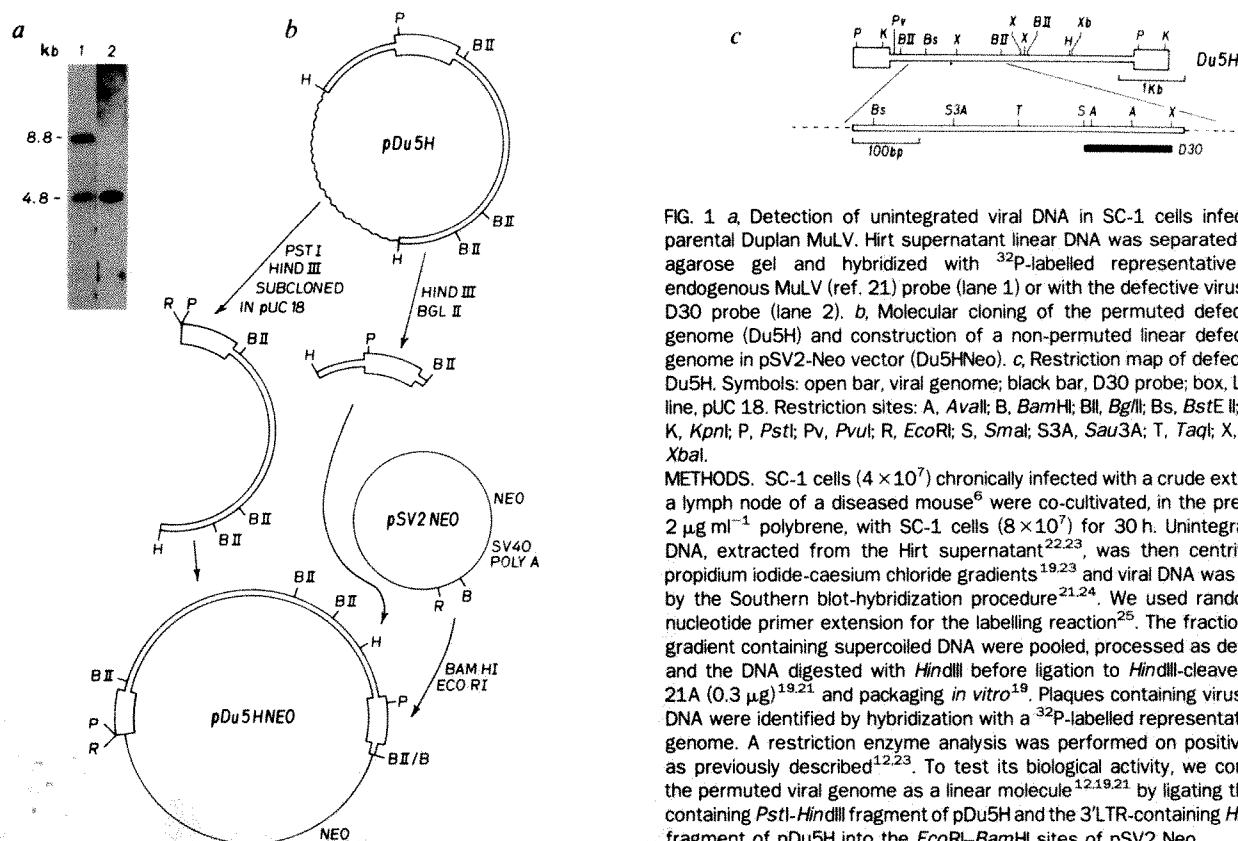


FIG. 1 a, Detection of unintegrated viral DNA in SC-1 cells infected with parental Duplan MuLV. Hirt supernatant linear DNA was separated on 0.7% agarose gel and hybridized with <sup>32</sup>P-labelled representative BALB/c endogenous MuLV (ref. 21) probe (lane 1) or with the defective virus-specific D30 probe (lane 2). b, Molecular cloning of the permuted defective viral genome (Du5H) and construction of a non-permuted linear defective viral genome in pSV2-Neo vector (Du5HNeo). c, Restriction map of defective virus Du5H. Symbols: open bar, viral genome; black bar, D30 probe; box, LTR; wavy line, pUC 18. Restriction sites: A, AclI; B, BamHI; BII, BglII; Bs, BstEII; H, HindIII; K, KpnI; P, PstI; Pv, PvuI; R, EcoRI; S, SmaI; S3A, Sau3A; T, TaqI; X, XhoI; Xb, XbaI.

METHODS. SC-1 cells (4 × 10<sup>7</sup>) chronically infected with a crude extract from a lymph node of a diseased mouse<sup>6</sup> were co-cultivated, in the presence of 2 μg ml<sup>-1</sup> polybrene, with SC-1 cells (8 × 10<sup>7</sup>) for 30 h. Unintegrated viral DNA, extracted from the Hirt supernatant<sup>22,23</sup>, was then centrifuged on propidium iodide-caesium chloride gradients<sup>19,23</sup> and viral DNA was detected by the Southern blot-hybridization procedure<sup>21,24</sup>. We used random hexanucleotide primer extension for the labelling reaction<sup>25</sup>. The fractions of the gradient containing supercoiled DNA were pooled, processed as described<sup>23</sup> and the DNA digested with HindIII before ligation to HindIII-cleaved Charon 21A (0.3 μg)<sup>19,21</sup> and packaging *in vitro*<sup>19</sup>. Plaques containing virus-specific DNA were identified by hybridization with a <sup>32</sup>P-labelled representative MuLV genome. A restriction enzyme analysis was performed on positive clones, as previously described<sup>12,23</sup>. To test its biological activity, we constructed the permuted viral genome as a linear molecule<sup>12,19,21</sup> by ligating the 5'LTR-containing PstI-HindIII fragment of pDu5H and the 3'LTR-containing HindIII-BglII fragment of pDu5H into the EcoRI-BamHI sites of pSV2 Neo.

linear molecules were further purified by agarose gel electroelution and a crude restriction map was obtained (data not shown). *Hind*III cleaved the defective DNA once, but did not cleave the most abundant species of non-defective viral DNA.

To clone the defective viral genome, supercoiled viral DNA was cleaved with *Hind*III and ligated into bacteriophage Charon 21A. Eight clones with an insert of 4.3 kb (one long terminal repeat, LTR) or 4.8 kb (two LTRs) were purified and their inserts subcloned in pUC 18. They all had a similar restriction map which was identical to the map derived from the uncloned Hirt DNA (Fig. 1c). One of these clones (Du5H) was selected and studied further. Several DNA fragments derived from Du5H were subcloned and used as probes to hybridize to the original Hirt DNA. Probe D30, derived from the 137-base pair (bp) *Sma*I-*Xho*I *gag* fragment (Fig. 1c), hybridized to the 4.8-kb species. But it did not hybridize to the 8.8-kb species of the original Hirt DNA (Fig. 1a, lane 2), nor to several other non-defective viral genomes from Moloney, wild mouse neurotropic Cas-Br-E, amphotropic 4070, ecotropic Kaplan V13 radiation leukaemia virus (RadLV) and endogenous ecotropic BALB/c (N-CL-35) MuLVs (data not shown); this suggests that this region of the genome is unique.

To determine whether the presence of the defective viral genome was associated with the disease-inducing potential of this virus, we studied five virus-producing cell lines, cloned from a population of chronically infected SC-1 cells. Three of the lines produced viruses able to induce the same disease as the parental Duplan virus when inoculated into C57BL/6 mice. They also contained a 1.53-kb *Bgl*/II fragment that hybridized to the defective virus-specific D30 probe. The other two lines produced a non-pathogenic virus and did not contain this *Bgl*/II fragment (data not shown). This suggested a role for this defective virus in the disease.

To test the biological activity of the defective viral DNA more directly, it was reconstructed as a non-permuted genome in a selectable vector (Fig. 1b), transfected into SIM.R fibroblasts and rescued with a non-pathogenic helper endogenous RadLV (G<sub>6</sub>T<sub>2</sub>)<sup>12</sup>. Mice injected with this pseudotyped defective MuLV developed clinically apparent disease within 8–12 weeks, as with the crude virus extract (Table 1). Mice inoculated with the helper (G<sub>6</sub>T<sub>2</sub>) alone were free of disease seven months after inoculation. The functional assay for T-lymphocyte response to the mitogen concanavalin A demonstrated that [<sup>3</sup>H]thymidine incorporation was suppressed in spleen cells from mice injected with Du5H

$\rightarrow U3$   
1 AATGAAGACCCACCATAAGGCTTAGCAAGCTAAGCTGCAATGCGCATGCGCCATCTTCCAGGCATGGAAAAATACCAAGACGCTG  
81 ATGTTCTTCAGAAAAACGAAGCAATAGGAGGATACAGAGGCTGGAAGATACGCGGACTAAGGCCAAACAGGATATCTTTGCG  
161 TTAGGACATAGGGCCCCCGGCCAGGGCCAGGAACAGGTGCTCCGCAAGAAATAGCTTAAACACAAACAGCTTTCAAGAGACG  
241 CAGAAACTGTCTCAAGGTTCGCCAGATGACCTGGGCAATCAACCCCAAGGCTCATTTAACTAACCAATACGCTGCGCTTG  
  
 $U3 \rightarrow R$   
321 TCGCTTCTGTATCCCGGCTTATTGTCGCCAGCTCTATAAAAAAGGTAAGAAACCCCAACCTCGGCCGCCAGCTCTCCGA  
  
 $R \rightarrow U5$   
401 TAGACTGAGTCGCCCGGGTACCGCTGTATCCAAATAAGGCTCTTGTGCTTGCACTCGGATCTGGTCTCGTTGATCTCTTG  
  
 $U5 \rightarrow$   
481 GGAGGGTCTCTCAGAGTGATTGACTGCCAGCTTGGGGCTCTTCTTTGGGGCTGCTCGGGATTTGGAGAACCCCGC  
  
*Pvu I*  
561 CCCAGGGACACGACCCACCGCTCGGGAGTAAGCTGCCACCAATCTTTTGTCTCCATCTGCTCTTTGTGGCTGTGT  
641 GTGTGCGGGCATTAATGTTTGGCGCTGGCTGTACTAGTTAGCTACATAGACTCTGATCTGGGGCTCCGTGGAGAAA  
721 CTGACGAGTTTCGGAATCTCCGACGACGCTTGAGACAGCTCTCAGGGGCATGCGGCGCTGAGTGGCCCAATCAGTA  
801 ATGTCGCAAGCTCTGACCACTGCGGACTATTTGGGCGCCCTCTTTCTTGGGAGGAGTACGTGGTATTTTAACTTTGCGTT  
881 TTGCGGAAATCGCGCGCGGCTCTGTCTGTCTGACTGTTGTTTGTCTTTTGTCTGTGCTTTTGTGTTTGGCGCGT  
  
 $p15$   
Met Gly Gln Thr Val Thr Thr Pro Leu Ser Leu Thr Leu Asp His Trp Lys  
961 TCTAAAAAT ATG GAC CAG ACC GTA ACC ACT CTT TTA AGT CTG ACC CTT GAC CAC TGG AAA  
  
 $\rightarrow GAG$   
Asp Val Gln Cys Ile Ala Ser Asn Gln Val Asp Val Lys Arg Arg Arg Trp Val Thr  
1021 GAC GTC CAG TGC ATT GCG TCC AAC CAG TCC GTC GAT GTC AAG AGC AGA CCG TGG GTC ACC  
  
Phe Cys Thr Val Trp Pro Ser Phe Asp Val Gly Trp Pro Leu Asp Gln Gly Thr Phe Asn  
1081 TTC TGC TCT GTC GAG TGG CCA ACT TTC CAT GTG GGG TGG CCA CTA GAT GGT ACT TTT AAT  
  
Leu Asp Ile Ile Leu Gln Val Thr Trp Gly Gln Ala Val Trp His Pro Lys His Pro Trp Val Thr  
1141 TGT GAC ATT ATT TTA CAG GTT AAA TCT AAG GTG ACT TGT CCG CCG CAC GGA CAC CCG  
  
Asp Gln Val Pro Trp Ile Val Thr Trp Gly Gln Ala Val Trp His Pro Lys His Pro Trp Val Thr  
1201 GAT CAG GTC CCA TAT ATC GTC ACC TGG GAG GCT CTT GTC TAT CAC CCG CCG CCG TGG GTC  
  
Lys Pro Phe Val Ser Pro Lys Pro Phe Pro Thr Thr Thr Leu Pro Phe Ser Pro Pro Gly  
1261 AAA CCG TTT GTC TCT CCA AAA CTT TTT CTT TTA TCG ACA CTT CCC TTT TCC CCG CCG GGT  
  
 $p15 \rightarrow p12$   
Pro Ser Ala His Pro Pro Ser Arg Ser Asp Leu Thr Thr Thr Ala Ile Pro Ser Lys Pro  
1321 CCT TCT GCA CAT CCT CCG TCC CGG TCT GAG CTT TAC ACT GGC CTT ATC CCG TCT AAA CCG  
  
Pro Lys Ser Arg Val Leu Thr Pro Asn Gly Gly Pro Leu Ile Asp Leu Leu Thr Glu Asn  
1381 CCT AAG TCC CCG GTT CTC CTT CTT AAC GGC GGA CTT CTC ATT GAC CTT CTC ACA GAG AAC  
  
Leu Pro Asn Leu Pro Pro Leu Ser Lys Gly Pro Val Lys Lys Arg Arg Pro Pro Arg  
1441 CTC CCT AAC CTT CTT CTT CTG TCA AAG GGA CCA GTT AAG AAG AAG CCG CCG CCA CCG CCG  
  
Arg Tyr Ser Pro Pro Pro Asn Pro Met Glu Ser Arg Val Arg Gly Arg Arg Asp Pro Cco Ala  
1501 AGG TAT TCT CCC CCT AAT CCC ATG GAG TGT CTA GCG GGG GGA AGG AGA CAG CTT CCC GCA  
  
 $p12 \rightarrow p30$  Xho I  
1561 GCG GAC TCC AAC TCC TCC CAG GCA Phe Pro Leu Arg Met Gly Gly Asp Gly Gln Leu Gln  
  
Tyr Trp Pro Phe Ser Ser Ser Leu Tyr Asn Trp Lys Asn Asn Asn Pro Thr Ser Phe Ser  
1621 TAT TGG CCG TTT TCC TCC TCC GAG TTA TAC AAT TGG AAA AAT AAT AAC CTT TCC TTT TCT  
  
Glu Asp Asp Gly Lys Leu Thr Ala Ile Glu Thr Ser Val Leu Thr Thr Cys Gln Thr Thr Thr  
1681 GAA GAT CCA GGT AAA TTG ACC GCC TTA ATT GAG TCT GTC CTC ACC ACC CAC CAA CCC ACC  
  
Tyr Asp Asp Cys Gln Gln Leu Leu Gly Trp Leu Thr Gly Gln Gly Gln Lys Gln Val Arg  
1741 TGG GAC GAC TGT GAG CAA TTG TTG GGG ACT CTG CTG ACA GAG GAA GAA AAG CAG CCG GTT

1801 Leu Leu Glu Ala Arg Lys Ala Val Arg Gly Asn Asp Gly Arg Pro Thr Glu Lys Pro Asn  
CTC TCA CAG GGC AGA AAG GCA GTC CGG GGC AAC CAT GGA GGC CCC ACC CAG TTG CCT AAT

1861 Glu Val Asn Ser Ala Phe Pro Glu Gly Arg Gly Arg Pro Asp Thr Asp Lys Thr Ser Thr Thr Glu Gly  
AGA GTC AAT TCC GCC TTC CCC CTT GAA CGT CCC CAT TGG AAT TAT TCA ACC CCT CAA GGT

1921 Arg Asn Ala Cys Leu Val Thr Tyr Arg Gln Leu Leu Ala Gly Leu His Asn Ala Gly Arg  
AGG AAC CAC CTA GTT CTC TAT CGC CAG TTG CTC TTA GCG GGT CTC CAG AAC GCG GCG AGA

1981 Ser Pro Thr Asn Leu Ala Lys Val Lys Arg Ile Thr Gln Gly Pro Asn Gly Ser Pro Ser  
AGC CCC AAG AAT TTG CGC AAG GTA AAG GGC ATA CAG GGC CCA CCA CAG TCT CCC TCA

2041 Ala Phe Thr Leu Glu Arg Leu Lys Glu Arg Arg Arg Thr Thr Pro Tyr Asp Pro Glu Asp  
CGC TTT TTA GAG AGA CTC AAG GAG GGC TAT CGC AGA TAC TCT CTT TAT CAG CCT GAG GAC

2101 Pro Gly Gln Glu Thr Asn Val Ser Met Ser Phe Ile Thr Gln Ser Ala Pro Asp Ile Gly  
CCA GGG CAA GAA ACC AAT GTG TCT ATG TCA TTC ATT TGG CAG TCT GCG CGG GAT ATC GGG

2161 Leu Thr Leu Glu Arg Asn Leu Asp Lys Arg Ser Lys Thr Leu Gly Asp Leu Val Arg Gln  
CGA AAG TTA GAG CGG TTA GAA GAT TTA AAG AGC AAG ACC TTA GGA GAG TTA GTG AGG GAA

2221 Gln Glu Lys Ile Phe Asn Lys Arg Gln Thr Thr Pro Glu Glu Arg Gln Glu Arg Ile Arg  
ACT GAA AAG ATC TTT AAT AAG CGA GAA AAG CCG GAA GAA GAA GAA CGT ATC AGG AGA

Glu Thr Glu Glu Lys Glu Glu Arg Arg Arg Ala Glu Asp Glu Arg Gln Glu Lys Glu Arg  
2281 GAA ACA GAG GAA AAA GAG CAA CGC COT AGC GAG GAG GAT CAG CAG AGG GAG AAA GAA AGA

p30 → p10

2341 Asp Arg Arg Arg Glu Glu Arg Met Ser Phe Leu Thr Val Val Thr Val Thr Thr Thr Thr  
GAC CGC AGG AGA CAT AGA GAG ATC AGC AAG TTC TTG GCG ACC ATG GTA GTT ACT GGT GAG CAG

2401 Gln Asp Arg Gln Glu Lys Glu Arg Arg Arg Thr Pro Gln Leu Asp Glu Arg Gln Cys Ala Tyr  
CAG GAT AGA CAG GCG GGA GAG CGA AGC AGG TTC CCA AAT TTT GAT GAG GAC CAA TGC GCC TAC

Cys Lys Glu Lys His Thr Arg Ala Lys Asp Cys Pro Lys Lys Pro Arg Gly Pro Arg Gly  
2461 TGC AAA GAA AAG GGA CAC TGG GCT AAA CAG TGC CGA AAG AAG CTT CAG GCG GCC CGA GAA

p10 → Xho I

2521 Pro Arg Pro Gln Thr Ser Leu Leu Thr Thr Glu Lys Asp \*\*\*  
CGC AGG CCC CAG ACC TCC CTC TCA ACC TTA GGT GAG TAG GAGGATCAGGTTCCAGGAGCCCCCAT

GAG →

2587 GATTGCTCCGAGATCTTGCTCGAAACGACGAGAACCAAGCAGACGACTTCACGACGACGCCCATCCCGAGCGCGGCACACC

Xho I

2647 TGGTATACCATCGGAGGAGCTTTTTCGACGAAGGACAGCAAAAGCTCGGACGACGAGTACGACGTGACAGCGAGTAAAT  
2767 CTCGCGGAGGCGCTCCGACATAGAACTGACCGCAGGACGCGCAATCGATCGCACTACCCCAAGCGCTTGAATATCGGAGA  
2827 AGGTGAAGGCGCAAAATGTTTATCTCGGACAGCGGATATGCTTTCCGCGAGGCCGCACTTCGATCGGAGGAATATCAAGAGGCG  
2907 GAGGTTGCTGACCTCAGAGGCGCAGCAAAATCAAAATTAAGAGGAGGATGCTTCGCTTTACTGAAGACTTTATCTCTGCTCT  
2987 AAAAGACTCACTAATTAATTCATCCGCGCGGATCAAAAGGGAAGAAGCTCTGCTGAAGCCGGGCAACTGTATGCGGACGAA  
3067 AGCGCGCCGACGAGGACCAATTAAGGACATTTCCGAAAGATTCACCACTTCTGCTAGAGGATCGACGCCCTATAGAGCCCT  
3147 CCCAAGAAGCCAAAGATCGGCGCGAGGCGCAAGTCACTCTGACTGCTTGCGAAGCACTCTCTCGCTGGGTGAAGGTTCTT  
3227 CCACAAAGACATGAGACTGCCAAAGTTCTACGCCCAAACTGCTTACGAAGAAATATTTCAATGCGGCCAA

Hind III

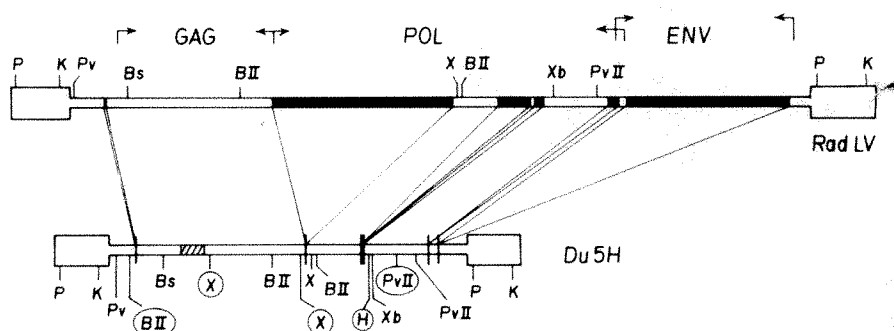
3307 GTATTGGGGCATGATAATGGCGCTGCTTCACTCCGACGAAGTCAGACAGTGCGGCAGTTGTGGGGATCGATTTGGAA  
3387 ACTGCATTGTGCTTACGCGGCCAGAGGTCAGGTCAAGTACGATAGAAGAAATGAACAGGACAAATCAAGAGCAATTTAAACCAAT  
3467 TAAGCTGTGACGCTGGACATAGAGACTGGGTCACTCTCACTCCCTTGCCCTCTACCGAGCGCGGAATCTCTCGGGGCCA  
3547 CATGAGCATTCGCTATGAGATTTCTGATAGAGCTTCTGATGAGGCTCCGAGGCTTCTGCAATTTTGATGATCTTGAAATGTCAAGAT  
3627 TACTTAATGCTTCTGCAAGCTCACTTACGAGGCTCCGAGGCTTCTGCAAGGAGGAGGAGGTTGCGAGCACTCTGGGCTCGAC  
3707 TAAGACACATGAGCTGGATACGACAGTATGACCAACCTCTTCCGAGGCTTCTGCAAGCTTCTTCAAAATCTCTTAAAG  
3787 TAAGACATTTAGACCCCGCTCGCAAGGACCTCAACGCTCTCTGCGACGACCCCACTGCTTCAAAATCTCTTAAAG  
3867 ATAAAGTTAAACCCCTGGGCGCCCTGATTTATAGCATCTTTGCTGAAGCGAGGCTCGGTCACAGCTCGACAGCCCT  
3947 CACACAGCTTTCAAGTTGTGACCGGCTGGTTATGAGAGATCTTTAAGCACTTCCCATGCTTCAAGTCAATTAATTCACUAA  
4027 TTATGGGCCCCCTTGATAATCACTTTTATTAATCTCACTTCTGGAGCCCTGATTATTCCTGGCTGGTCCGATCTTCTGAAGA  
4107 CAGCAAAATTTTGAATGTCAGGCGCTGGTTTGTACCCCAAGCATGACCAACCAATTAATGATATCCAGAGAGATGAA  
4187 ATCAGCTGAATAAAGATTTTATTCAGTTTCCACGAAGAGGGGGATGAAGAACGCCACAC.....

→ ITR U3

FIG. 2 Nucleotide sequence of defective Duplan Du5H virus. The complete nucleotide sequence is shown together with the predicted amino-acid sequence of the largest open reading frame. The position of sequences encoding Gag p15, p12, p30 and p10 proteins are indicated and the positions of a few restriction endonuclease sites are also shown.

and the resulting fragments were subcloned into pUC18 vector. Plasmid DNAs were prepared by the alkaline method<sup>19</sup>. The sequence was determined by the dideoxynucleotide method of Sanger<sup>26</sup>, using double-stranded supercoiled plasmid DNA as the substrate for the polymerizing enzyme (Sequenase, United States Biochemicals)<sup>27</sup>. Oligonucleotide primers were complementary to the pUC18 vector. Both strands of the viral genome were sequenced.

FIG. 3 Comparison of the restriction map of the defective Du5H viral genome with that of non-defective RadLV (refs 12, 13). Common restriction enzyme sites are indicated. Several large deletions are present in *pol* and *env*. The *gag* sequences are homologous in p15, p30 and p10. Symbols: white box, homologous; black box, deleted; hatched box, unique sequences. BII, *Bgl*II; Bs, *Bst*E II; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*I; PvII, *Pvu*II; X, *Xho*I; Xb, *Xba*I; Circled restriction endonuclease sites are sites present in Du5H, but not in RadLV.



(G<sub>6</sub>T<sub>2</sub>) MuLV, but not in mice injected with helper (G<sub>6</sub>T<sub>2</sub>) alone (Table 1). Serum IgM was also markedly elevated in the Du5H (G<sub>6</sub>T<sub>2</sub>) MuLV-inoculated diseased mice, as compared with the controls (Table 1). Therefore, the defective Du5H genome pseudotyped with a non-pathogenic helper MuLV was sufficient to induce the same disease as the crude Duplan extract, namely lymphadenopathy, splenomegaly, immune suppression and hypergammaglobulinaemia.

To study the structure of this defective viral genome in more detail, we obtained its nucleotide sequence (Fig. 2). Comparison of this sequence with that of other RadLVs (refs 12, 13) revealed several large deletions in *pol* and *env* and a relatively well conserved region in *gag* (Fig. 3). The LTR sequences are very similar to those of the endogenous ecotropic MuLV from C57BL/6 mouse<sup>12</sup>, diverging by only six point mutations in the region U3, one in R and three in the U5 region. They contain only one 99-bp direct repeat in U3. The sequences corresponding to *gag* p12 and to the D30 probe diverged the most from those of other MuLVs. Four large putative open reading frames could be recognized. Three (175, 173 and 170 amino acids; nucleotides 1,175–1,699, 3,241–3,759 and 3,469–3,979, respectively), correspond to part of *gag*, part of *pol* and part of *pol-env*, respectively. The fourth and largest open reading frame (nucleotides 802–2,559) has an ATG codon corresponding to the N-terminus of Gag p15 (nucleotide 970) and continues to p15, p12, p30 and p10. The p15, p30 and p10 showed respectively 81%, 95% and 95% identity to the corresponding amino-acid sequence of AKR MuLV (AKV) (ref. 14), the sequence in the data banks that was closest to it. The Du5H p12 is different from VL3-RadLV (ref. 13) or AKV (ref. 14) p12, however, being shorter by eight amino-acid residues and diverging most notably in its 50-amino acid C-terminus. This unique amino-acid sequence is rich in prolines and has many basic amino-acid residues. A computer search of current entries in sequence libraries revealed that the Du5H Gag p12 C-terminal region had only 40–50% identity with Gag p12 protein of other murine retroviruses, and was not identical to any other non-retroviral sequences in the database.

The pathogenic virus present in the crude Duplan RadLV extract is a unique defective retrovirus capable of inducing a severe immunodeficiency disease after rescue with a non-pathogenic helper ecotropic RadLV. This virus represents the second example, in addition to the variant defective FeLV<sup>4,5</sup>, of an immunodeficiency-inducing retrovirus that is replication-defective. Our results re-emphasize<sup>5</sup> the need to search for similar pathogenic replication-defective variants in other immunodeficiency diseases, including AIDS, and suggest that the pathogenic AIDS virus may also be a defective retrovirus.

The Duplan defective RadLV genome has deleted its *env* region and has a well conserved *gag* region with a unique p12 sequence encoding a putative novel protein. Because this p12-related protein is unique and because of the absence of any other unique long open reading frame, this *gag* region is probably important in the pathogenesis of the disease. Only one

retrovirus, the avian osteopetrosis virus, is known to contain its chief determinant of pathogenicity within its *gag* region<sup>15</sup>. The origin of the unique and shorter p12-related sequence of Duplan defective RadLV remains unclear. It may be derived by recombination between genomic sequences and a primary non-defective RadLV<sup>12</sup>, or it might have evolved by multiple modifications (point mutations, deletions, substitutions and so on of a known *gag* p12 sequence).

The mechanism by which this defective virus induces immunodeficiency remains to be determined, but the similarities of this syndrome with human AIDS are striking. Both viruses induce polyclonal B-cell proliferation, manifested by lymphadenopathy, splenomegaly and hypergammaglobulinaemia, as well as severe immunodeficiency (involving both T-lymphocyte and B-lymphocyte functions), enhanced susceptibility to infections and terminal B-cell lymphomas<sup>2,3,6–11,16</sup>. The presence of T lymphocytes was found to be essential to the development of the murine disease<sup>17</sup>. Therefore, a common perturbation or perturbation of a common pathway may underline this murine syndrome and human AIDS. Further study of this system may lead to better understanding of the pathogenesis of retrovirus-induced immunodeficiency diseases, including AIDS. □

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# Alternative splicing of human dystrophin mRNA generates isoforms at the carboxy terminus

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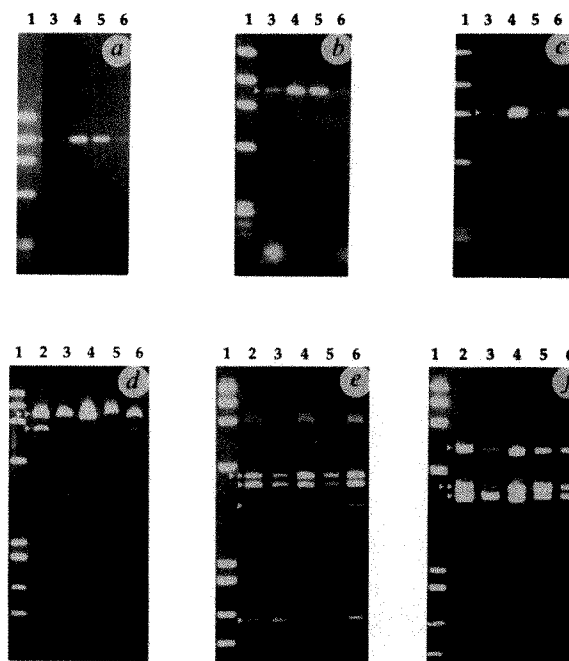
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**DYSTROPHIN** is the protein product of the Duchenne/Becker muscular dystrophy locus. It has a relative molecular mass of 427,000 and is encoded by a large RNA transcript processed from more than 65 exons spread over two million base pairs of the human X chromosome<sup>1-5</sup>. We have used the polymerase chain reaction<sup>6</sup> to see whether any of these exons are used alternatively in the different tissues that express dystrophin. As reported for rat dystrophin<sup>7</sup>, we find that the first exon of the human dystrophin transcript is different in brain and muscle, indicating that dystrophin expression could be differentially regulated in these tissues by usage of distinct promoters. The 3' end of the dystrophin transcript can be alternatively spliced to create numerous isoforms differing at their carboxyl domains; this is the only domain of dystrophin that does not share any similarity with the related cytoskeletal  $\alpha$ -actinins<sup>3</sup>. These alternative transcripts yield dystrophin molecules which may interact with different proteins of the tissues expressing dystrophin.

Northern blot analysis of dystrophin messenger RNA from different tissues did not reveal heterogeneity of the transcript in any given tissue, or from one tissue to another (refs 8, 9 and unpublished observations), although minor size variations are difficult to detect. Western blots reveal a predominant protein band corresponding to  $M_r$  427K for dystrophin<sup>1</sup>. Smaller forms are detectable however, and these could represent immunocross-reactive proteins, partial proteolytic breakdown products or isoforms of dystrophin<sup>1,10</sup>. One of these smaller forms ( $M_r$  ~405K) of dystrophin is predominant in smooth muscle of mouse<sup>10</sup>. To test whether alternative splicing is responsible for these differences in protein size, we used the polymerase chain reaction (PCR) (ref. 6) to screen different regions of the human dystrophin mRNA for lost or gained exons. Twelve pairs of primers, derived from the dystrophin complementary DNA sequence<sup>3</sup>, were designed to amplify overlapping segments of ~1 kilobase spanning the entire dystrophin coding sequence. Amplified fragments were visualized directly on polyacrylamide gels or on agarose gels which were Southern blotted and hybridized with cloned skeletal muscle dystrophin cDNA. Any amplified segment of a size not predicted from sequence analysis would indicate a variation resulting from alternative splicing. All segments were amplified from single-stranded cDNA prepared from four different human tissues known to express dystrophin: skeletal muscle (psoas or leg muscles), cardiac muscle, smooth muscle (stomach or aorta) and brain. Tissues expressing lower levels of dystrophin<sup>11</sup> were not analysed.

The sizes of the amplified fragments corresponding to segments 2 to 10 were as predicted from the sequence of dystrophin cDNA from skeletal muscle<sup>3</sup> and were identical for all four tissues tested (Fig. 1b and c). The predicted fragments for the other segments were not uniquely observed in each tissue. The amplified fragment corresponding to segment 1 was found in skeletal, cardiac and smooth muscle, but not in brain tissue (Fig. 1a). This result indicated that the sequence of one of the two primers used to amplify this 5' segment could be absent in the major form of the brain dystrophin transcript. Subsequent blotting of the gel and hybridization with a dystrophin cDNA probe revealed traces of the expected fragment in the brain reaction, possibly as a result of the smooth muscle content of brain (data

not shown). The fragment expected for segment 11 was found in all the tissues we tested, but a smaller and less abundant fragment was also co-amplified (Fig. 1d). The pattern of fragments obtained for segment 12 was more complex. To map this rearranged region more precisely, we amplified segment 12 as two smaller overlapping sub-segments. The amplification of the 5' sub-segment, 12a (Fig. 1e), gave the expected fragment and three other fragments that were smaller by ~40, 100 and 330 base pairs (bp). The amplification of the 3' sub-segment, 12b



**FIG. 1** PCR amplification of six segments of the dystrophin transcript. Amplified products were separated on 1.2% agarose gels (a-c) or on 4% polyacrylamide gels (d-f). Lane 1 is  $\Phi$ X174 replicative form DNA/*Hae*III-cut standard (BRL); lanes 2, 3, 4, 5 and 6 are amplification products of human fetal aorta, brain, heart, leg and stomach tissue respectively. a, Amplification product of segment 1 (position relative to the published cDNA sequence 141-1,207); b, segment 5 (3,770-4,771); c, segment 8 (6,602-7,462); d, segment 11 (9,395-10,342); e, segment 12a (10,288-10,840); f, segment 12b (10,811-11,350). The fragments expected from the skeletal muscle cDNA sequence are indicated by a single white arrow, or a double white arrow when more than one fragment is apparent. The expected fragment of segments 1, 5 and 8 (1,067, 1,002 and 861 bp respectively) is observed in all tissues, except for segment 1 in brain (Fig. 1a, lane 3). In d, a 948-bp fragment is detected along with an unexpected fragment of ~800 bp in length. The lower band appears to be more prevalent in aorta (lane 2) than in other tissues. In e, the expected amplified fragment of 553 bp is seen from all tissues, along with three other fragments of ~510, 450 and 225 bp respectively (material in lane 5 was obtained from a separate experiment). The 553-bp fragment is more prevalent in leg and cardiac muscle, whereas the most prevalent fragment from smooth muscle and brain is 510-bp. The 450- and 225-bp fragments also appear more in smooth muscle and brain than in striated muscles. In segment 12b (f) there are three amplified products (700, 540 and 510 bp). The more prevalent fragment in smooth muscle and brain samples is 510 bp, whereas the expected 540-bp fragment is more prevalent in cardiac and skeletal muscle. We are analysing the 700-bp fragment at present: it could represent use of a new 160-bp exon or simply be an artefact of the PCR, which is supported by the fact that it is not seen under different electrophoresis conditions.

**METHODS.** Total mRNA was isolated by guanidine isothiocyanate extraction<sup>15</sup> and was reverse-transcribed into single-strand cDNA using oligo (dT) (18-25) as primer and reverse transcriptase from Moloney murine leukaemia virus (BRL). Samples were extracted with phenol/chloroform and ethanol-precipitated. The PCRs used 30-mer oligonucleotides (Biopolymers Laboratory, Howard Hughes Medical Institute at Harvard Medical School) which had been purified by electrophoresis on a 20% denaturing polyacrylamide gel. PCR conditions were as recommended (Cetus Corporation), using ~40 ng cDNA and 1  $\mu$ g of each primer. Annealing and primer extension were at 72°C, to minimize artefactual priming, and the denaturation step was at 94°C.

(Fig. 1f), gave three fragments, one of the predicted size, one ~35 bp smaller, and another ~160 bp larger. These results indicate that there are three distinct regions involved in potential alternative splicing in the last 2 kb of the coding sequence. The relative proportions of each fragment within a single sample in segments 11, 12a and 12b differed from tissue to tissue. Analysis of the relative abundance of the fragments indicated that the fragments predicted from the 'skeletal muscle' sequence were indeed more abundant in striated muscles (skeletal and cardiac), whereas the other fragments were usually more abundant in smooth muscle and/or brain.

To investigate the absence of amplification product for segment 1 in brain, we isolated three brain cDNA clones using a 5' skeletal muscle cDNA probe. The sequence of one clone matched the skeletal muscle sequence over the second and third exon, but differed over the entire length of the first exon (Fig. 2a), whereas the sequence of two clones matched the sequence of skeletal muscle cDNA<sup>3</sup> perfectly. The sequence of the alternative first exon indicates that this exon has an in-frame stop codon, followed further downstream by an initiation codon in-frame with the rest of the coding sequence. The alternative first exon would therefore encode only 2½ amino acids (Fig. 2a), replacing the 10½ amino acids of the muscle first exon. To confirm that an alternative first exon is used in brain tissue, we used a primer specific for the alternative exon for the PCR amplification of the corresponding transcript, along with parallel amplification of the 5' end of the skeletal muscle transcript (Fig. 3a and b). We found the alternative first exon in the main form of the brain transcript but also in transcripts of skeletal, cardiac and smooth muscles (Fig. 3b). This same exon has been found in rat brain transcripts<sup>7</sup>. It is striking that the 5' (apparently) untranslated

portion of this exon is highly conserved in human and rat (99% homology; Fig. 2a). In comparison, the 'skeletal muscle' first exon is only 80% homologous between human and mouse<sup>2</sup>. A different first exon in brain implies that there are alternative promoter and regulatory sequences in brain and muscle, which could put expression of dystrophin in these tissues under different control systems.

We investigated the multiple fragments obtained by amplification of segments 11, 12a and 12b by direct sequencing with nested primers. In addition, the sequence of several cDNA clones isolated from skeletal muscle and brain cDNA libraries was determined over the regions involved in differential splicing. In all cases, fragments smaller than those predicted for 'skeletal muscle' resulted from loss of one or more exons, with no replacement of new nucleotide sequences (Fig. 4). The loss of exons in segment 12a maintained the reading frame but the loss of exons in segments 11 and 12b modified the reading frame, resulting in potential coding for new amino-acid sequences. Interestingly, the exon loss in segment 11 results in deletion of the entire carboxyl domain of dystrophin, and the 5' border of the deletion corresponds exactly to the end of the sequence similarity of dystrophin with the carboxyl domain of  $\alpha$ -actinin<sup>3</sup>. Such dystrophin molecules would be formed only by the three domains that are evolutionarily conserved with  $\alpha$ -actinin and might represent an ancestral form of the molecule.

The sequence of skeletal muscle and brain cDNAs confirmed the existence of the most abundant forms of transcript in the two tissues (Fig. 4). However, one skeletal muscle clone has been described as containing a portion of a putative unspliced intron<sup>3</sup> (the position of the splice site was misindicated in Fig. 1 of this ref.) and one of the brain cDNA clones has the same

FIG. 2 5'-Terminal sequence of a brain cDNA clone, including the 'brain' first exon and putative intron sequence found in muscle and brain cDNAs. Exon boundaries are indicated by black triangles and relevant stop codons are underlined. a, Sequence of the first, second and part of third exon of human brain cDNA. The equivalent sequence from rat<sup>7</sup> is shown for comparison. Mismatches are boxed. An ATG codon in frame with the coding sequence is found 7 nucleotides upstream from the first exon border and is preceded by a stop codon in the same reading frame. b, Sequence of the 3' end of two independent cDNA clones containing the same putative intron. The position where the sequence diverges from the skeletal muscle sequence is immediately followed by a typical donor splice site (boxed). The position of divergence is also the 5' border of the coding sequence deletions observed in segment 12a (see text). Numbering is according to the skeletal muscle cDNA sequence<sup>3</sup>. cDNA library construction and screening have been described<sup>2,8</sup>. Both strands of the inserts subcloned into pBR322 or bluescript vectors were sequenced with the Sequenase kit (USB) using universal primers and cDNA-specific oligonucleotide primers.

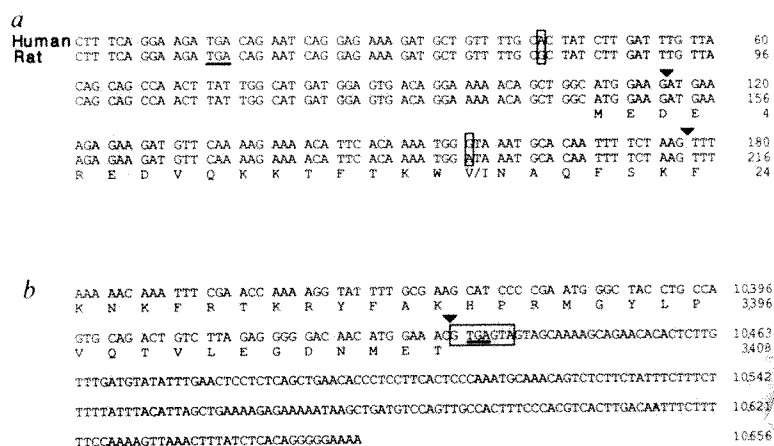


FIG. 3 Dystrophin transcript amplification using primers from the alternative first exon and from the putative 3' intron. Amplified fragments were separated on polyacrylamide gels. Lane 1 shows  $\phi$ X174 replicative form DNA/*Hae*III-cut standard size markers, as in Fig. 1. Lanes 2, 3, 4, 5 and 6 contain cDNA from human fetal aorta, brain, cardiac, leg muscle and stomach respectively. a and b, Amplification with a 5' primer from the skeletal muscle first exon gives no detectable fragment in the brain reaction (lane 3, panel a), whereas amplification with a 5' primer from the alternative first exon produced the expected 750-bp fragment (white arrow) not only in the brain reaction (lane 3, panel b) but also in the cardiac and leg muscle reactions (lanes 4 and 5, panel b). c, Coamplification of segment 12a with two different 3' primers. One 3' primer is from the dystrophin coding sequence (as in Fig. 1) and the other 3' primer is from the putative intron sequence (positions 10,570 to 10,599 in Fig. 2b). Amplification with the intron 3' primer produced the expected 312-bp fragment (dark arrow) in addition to the four fragments obtained with the coding sequence primers (white arrows; compare with Fig. 1e). RNA preparation, cDNA synthesis and PCR amplification are described in the legend to Fig. 1.

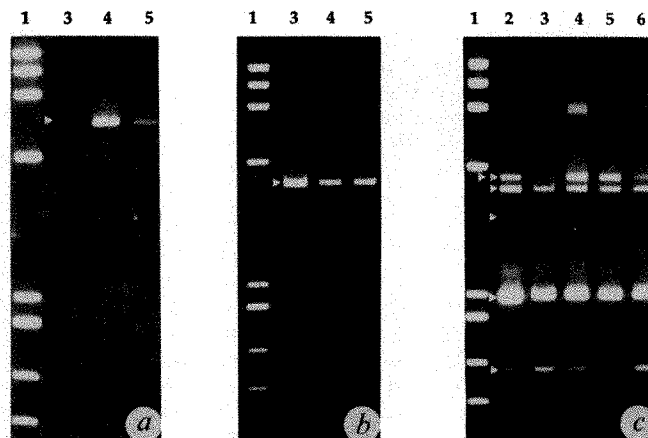
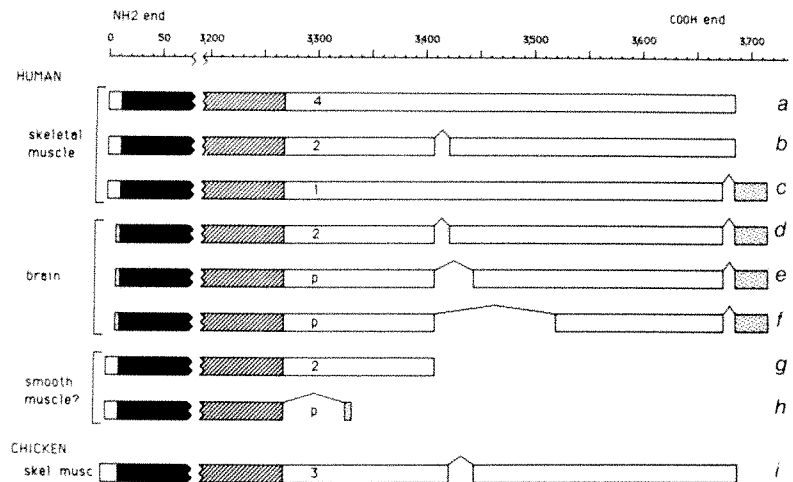


FIG. 4 Summary of possible protein forms encoded by the transcript variants described in this study. N- and C-termini of the dystrophin proteins are represented by bars labelled *a* to *i*. The black and hatched bars represent portions of the domains homologous to the amino and carboxyl domains of dictyostelium  $\alpha$ -actinin respectively. Dotted bars represent amino-acid sequences differing from the published dystrophin sequence. The large open bar represents the carboxyl domain of dystrophin. The numbers indicated in these open bars are the numbers of cDNA clones isolated from brain and skeletal muscle cDNA libraries encoding the corresponding form of the carboxyl domain. *p*, Forms that have been identified solely from the sequence of fragments amplified by PCR. The different forms are not strictly tissue-specific and other forms corresponding to combinations of differentially spliced exons are possible. The tissues in which a particular form is thought to be predominant are shown on the left; 'skeletal' refers to both skeletal and cardiac muscles. The scale is numbered by amino-acid residues and is shown at the top of the diagram. The description of the skeletal muscle form of the chicken dystrophin (*i*) is from Lemaire *et al.*<sup>14</sup>. The different rearrangements at the origin of the forms (*a* to *i*) is as follows; *a*, human skeletal muscle consensus form; *b*, 39-bp deletion (positions 10,432 to 10,470); *c*, 32-bp deletion (11,223 to 11,254); *d*, 'brain' first exon and 39- and 32-bp deletions; *e*, 'brain' first exon, 32-bp deletion and 105-bp deletion (10,432 to 10,536); *f*, 'brain' first exon, 32-bp deletion and 330-bp deletion (10,432 to 10,761); *g*, putative intron sequence (starting at position 10,432, see Fig. 3); *h*, 167-bp deletion (10,016 to 10,182); *i*, 66-bp deletion (positions 10,471 to 10,536 relative to the human cDNA map).

**METHODS.** Cloning and sequencing of the cDNA clones is described in the legend to Fig. 2. Fragments from PCR amplification of segments 11, 12a and 12b were gel-purified before sequence determination. As the different fragments were present in all tissues, they were prepared from the tissue where they were most abundant. The fragments detected in segments 12a and 12b were purified from reactions using brain and heart material and



fragments from segment 11 from aorta. Typically, 5 PCR reactions were pooled and ethanol-precipitated with 0.1% SDS and tRNA as carrier and electrophoresed on 1% low-melting point agarose gels or 4% polyacrylamide gels. Fragments were cut from the gels and either directly purified and concentrated with the GeneClean kit (Bio101) (agarose gels) or electro-eluted in dialysis bags and then purified and concentrated with the GeneClean kit (polyacrylamide gels). After boiling, fragments (100 ng) were directly sequenced with the Sequenase kit ('dGTP' mixes, USB) and S35-dATP (>1,000 Ci mmol<sup>-1</sup>, Amersham). In some cases the concentration of the respective deoxynucleotides in the termination mixes was increased from 8 to 32 mM to maximize the elongation of molecules over the size range of interest (between 100 and 200 nucleotides). The entire sequencing reaction was run on a 6% polyacrylamide gel and exposed for 7 days.

intron sequence (Fig. 2b). Interestingly, the splice site starts at the same position as the deletion variations of segment 12a. Two explanations would account for the finding of two independent clones, apparently representing the same intermediate form of splicing. The splicing machinery could pause at this key intron, resulting in a substantial accumulation of the intermediate form. Alternatively, the intron could be part of a mature transcript. The coding sequence of this transcript terminates at a TGA stop codon contained in the putative donor splice site (see Fig. 2b). The transcript could therefore encode a dystrophin with the last 277 amino acids missing (7.5% of the entire protein), a size corresponding to the smaller form of dystrophin seen on western blots<sup>10</sup>. To confirm the natural occurrence of the putative unspliced intron found in the two cDNA clones, we made a primer from the intron sequence and used it with the previous primers to amplify segment 12a. We detected the predicted 312-bp fragment representing this unusual transcript in all tissues (most prominent in the aorta sample), together with the four fragments seen previously (Fig. 3c). The presence of this 312-bp form in all tissues could be explained either by the smooth muscle content in these tissues, or because it is expressed in all tissues. The fact that we found only two of the cDNA clones out of eleven indicates that this alternative form is probably a minor species in brain and skeletal muscle (the strong intensity of the 312-bp band does not necessarily reflect the relative abundance of this form as the final PCR yield also depends on primer efficiency).

The alternative isoforms of dystrophin we have detected in the amino and carboxyl domains may not represent all possible forms of dystrophin in the cell as the PCR procedure can detect only those forms for which sequence is available. Analysis of cDNA clones from muscle and brain indicates that undetected forms are minor compared with those we present, otherwise they would have been isolated as cDNA clones. The first three domains of dystrophin are similar to those of the cytoskeletal proteins  $\alpha$ -actinin and spectrin<sup>3,12,13</sup> and the terminal 600 amino

acids of dystrophin have been subdivided into two domains on the basis of their cysteine content and similarity to  $\alpha$ -actinin<sup>3</sup>. The variation in the primary sequence (Fig. 4) of dystrophin affects the last domain, which has no counterpart in  $\alpha$ -actinin. It is interesting to speculate on the biological significance of the different forms of this domain in view of the surprising degree of conservation between the human and chicken dystrophin over the last 650 amino acids of the protein<sup>14</sup>, which implies that this terminal domain may interact with other highly conserved structural proteins. Here we have shown that this domain is alternatively spliced, resulting in a potential to encode different polypeptides. Each difference (Fig. 4) is generated by selective removal of exons at three key splice junctions. If the carboxyl domain anchors dystrophin to the inner surface of the plasma membrane through integral membrane proteins, then the discovery of tissue-specific isoforms indicates that proteins which interact with dystrophin could differ between tissues in which dystrophin is expressed. □

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# Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells

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THE initiation of a specific immune response is believed to require not only activation through antigen-specific receptors on T cells and B cells but also antigen-independent interactions between accessory molecules<sup>1</sup>. One such molecule is LFA-1, which enhances the avidity of interactions between T cells and antigen-presenting cells<sup>2-4</sup>, and is possibly involved in signal transduction across the T-cell membrane<sup>5</sup>. Intercellular adhesion molecule-1 (ICAM-1), a surface glycoprotein of relative molecular mass ( $M_r$ ) 80,000–110,000, has been defined as a ligand for LFA-1<sup>6,7</sup>, and has been shown to participate in the interaction between T cells and monocytes<sup>8</sup>. The determination of the precise contribution of such accessory molecules to antigen presentation, however, is complicated by the need to analyse against a background of multiple molecular interactions. We have investigated the role of LFA-1/ICAM-1 interactions in antigen presentation directly by quantifying the contribution of ICAM-1 expression to T-cell stimulation using L-cell transfectants that co-express ICAM-1 and HLA-DR. In the case of transfectants expressing modest levels of HLA-DR, co-expression of ICAM-1 is critical for effective HLA class II-restricted and allospecific T-cell activation, pointing to an important role for ICAM-1 in the induction of T-cell responses.

Previous studies from our laboratory showed that L-cell transfectants expressing a high level of HLA-DR2 could present antigen efficiently to several antigen-specific, HLA-DR2 restricted T-cell clones without any apparent requirement for LFA-1/ICAM-1 interactions<sup>9</sup>. Some T-cell clones, however, could not be activated in this way, implying that the expression of other human products by the antigen-presenting cells (APC), in addition to HLA class II, was required for effective antigen recognition. Moreover, using L cells transfected with HLA-DR7 (LDR7) (ref. 10), which express levels of HLA class II similar to monocytes, we have been unable to detect any significant antigen or allospecific stimulation of freshly isolated T cells. We therefore investigated whether reconstitution of APC function could be achieved in this case by supertransfecting the HLA-DR7 transfectants with the accessory molecule ICAM-1.

A full-length complementary DNA clone encoding ICAM-1 (ref. 7) was subcloned into the SV40-based cDNA expression vector pJ3 $\omega$  (ref. 11) and used to transfect mouse Ltk<sup>-</sup> cells and L-cell transfectants expressing HLA-DR7 (LDR7) (ref. 10). After flow-microfluorometric sorting and single-cell cloning, cell lines expressing ICAM-1 (L-ICAM-1) and ICAM-1 plus DR7 (LDR7-ICAM-1) were obtained (Fig. 1). Both sets of transfectants bound the ICAM-1-specific monoclonal antibody RR1/1 (ref. 12), their level of expression being similar to that of the B-lymphoblastoid cell line PGF or fibronectin-adherent monocytes. Studies in murine systems have indicated that mouse L cells do not express their own functional ligand for mouse LFA-1 (ref. 13). The level of HLA class II expressed by the LDR7-ICAM-1 transfectants was indistinguishable from that of the parental cell line LDR7, this being  $\sim 1/10$  that observed for the LDR2 transfectants. The large difference in the levels of

HLA class II expressed by LDR7 and LDR2 cells results from the use of different expression vectors during subcloning of the respective cDNA clones used for transfection<sup>9,10</sup>.

Several studies have reported the failure of mouse L cells transfected with HLA products to serve as targets for alloreactive human cells<sup>14,15</sup>. The transfectants L-ICAM-1, LDR7 and

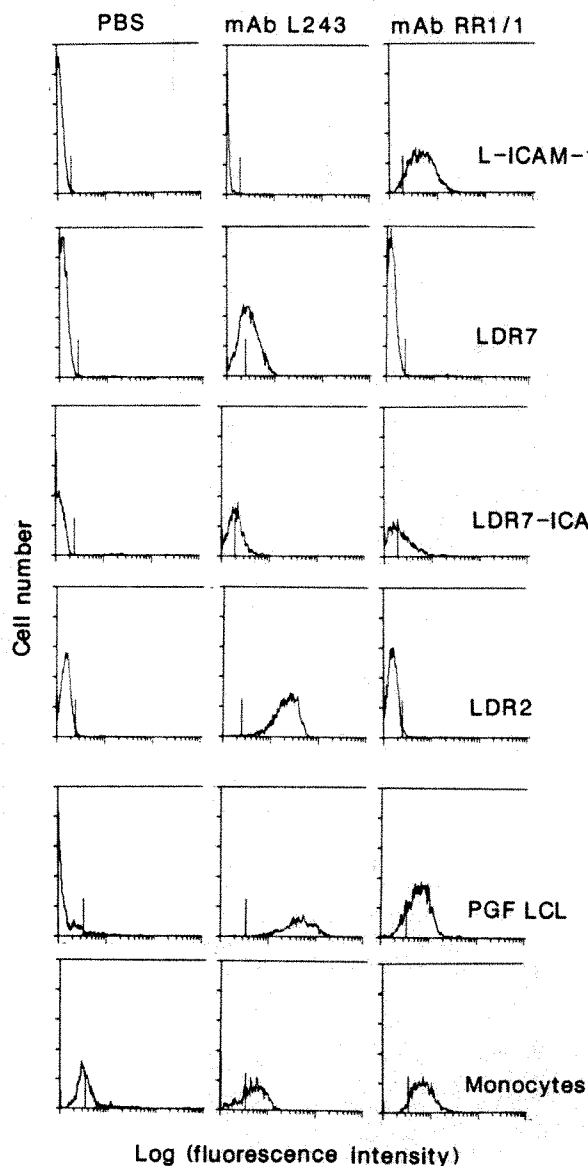


FIG. 1 Flow cytometric analysis of cellular HLA-DR and ICAM-1 expression. Cells were analysed for surface expression of HLA-DR (monoclonal L243) (ref. 17) and ICAM-1 (monoclonal RR1/1) (ref. 12), with rabbit anti-mouse immunoglobulin-FITC as second antibody, by flow cytometric analysis on a FACScan Analyzer (Becton Dickinson). The data are plotted as log fluorescence intensity (in arbitrary units) against cell number. Transfectants were compared with the HLA-DR2Dw2 homozygous B-lymphoblastoid cell line, PGF, and with peripheral blood monocytes prepared by overnight adherence of freshly isolated peripheral blood mononuclear cells to fibronectin-coated dishes. The level of HLA class II expression on monocytes varied among experiments and individuals; the data shown represent a population that falls within the limits of the observed range.

METHODS. Generation and characterization of the LDR7 and LDR2 transfectants has been described in detail elsewhere<sup>9,10</sup>. L-ICAM-1 and LDR7-ICAM-1 transfectants were generated by transfection of Ltk<sup>-</sup> cells and LDR7 cells respectively with an ICAM-1/pJ3 $\omega$  construct using the calcium phosphate precipitation technique. Plasmids encoding neomycin resistance (pSV2neo) or the herpes simplex virus thymidine kinase gene (pOPF) were cotransfected to allow selection of stable transfectants with Geneticin (Gibco) or hypoxanthine/methotrexate/thymidine, respectively. Drug-resistant colonies were pooled and established as stable lines before cloning.

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LDR7-ICAM-1 were therefore tested for cytotoxic allorecognition by JLMH1, a DR7-specific alloreactive T-cell line (Fig. 2a). At high effector/target ratios, the LDR7 transfectants showed only marginal specific lysis and no lysis of L-ICAM-1 cells was seen, indicating that neither molecule alone was sufficient to trigger significant effector function. The LDR7-ICAM-1 transfectants, however, served as efficient targets for JLMH1, the contrast with the targets expressing HLA-DR7 alone being most marked at low effector/target ratios. Cytolysis was inhibited by monoclonal antibodies (mAbs) against LFA-1 $\beta$  (the common  $\beta$ -chain of the LFA-1 family<sup>16</sup>, ICAM-1 (ref. 12), HLA-DR (ref. 17) and CD4 (Leu3a) (Fig. 2b). Thus, the inefficient allorecognition

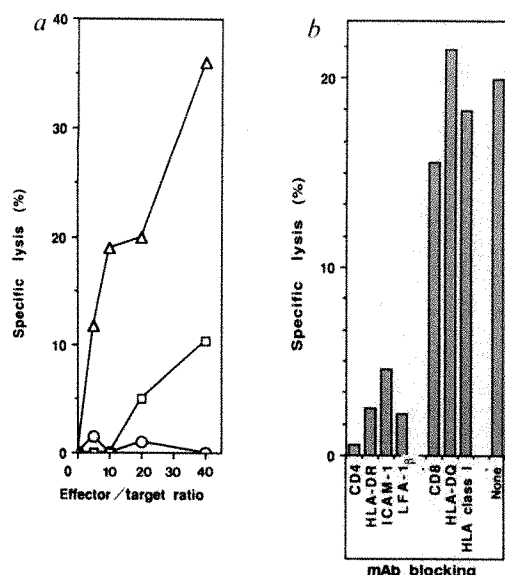


FIG. 2 Role of ICAM-1 in lysis of transfectants by cytotoxic T-cells. *a*, Target cells ( $1 \times 10^4$  per well) were labelled with  $^{51}\text{Cr}$  (Amersham) and cultured at the indicated effector to target ratios with JLMH1 T-cells in V-bottom wells for 7 h, at which time supernatants were counted in a Beckmann Gamma 4000 Counter. Each point represents the mean of triplicate cultures. JLMH1 is a long-term,  $\text{CD4}^+$ , alloreactive T-cell line, initially generated by stimulation of PBM cells from an HLA-DR4Dw4 homozygous individual with cells from an HLA-DR7 homozygous individual. The line is HLA-DR7-specific, recognizing all HLA-DR7 positive B lymphoblastoid cell lines but not HLA-DR1 or DR6 cell lines. Lysis of the HLA-DR7 homozygous B-LCL MANN was 83% (E/T=40). Circles, L-ICAM-1; squares, LDR7; triangles, LDR7-ICAM-1. *b*, Cytolysis of the LDR7-ICAM-1 transfectant was assessed at an effector/target ratio of 20 in the presence of various mAbs. Antibodies were added to cultures as a 200-fold dilution of purified immunoglobulin or ascites representing a final concentration of  $10\text{--}15 \mu\text{g ml}^{-1}$ . Monoclonals used: Leu3a (anti-CD4); L243 (anti-DR1 $^7$ ); RR1/1 (anti-ICAM-1)<sup>12</sup>; MHM23 (anti-LFA-1 $\beta$  chain)<sup>16</sup>; OKT8 (anti-CD8); Genox 3.53 (anti-HLA-DQw1); and W6/32 (anti-HLA class I).

of LDR7 was not due to the qualitative absence of a cell-type specific alloantigenic peptide, as has been suggested following the failure to observe allorecognition in other transfection systems<sup>15,18</sup>, but rather reflects a quantitative dependence of T-cell recognition and/or effector function on accessory molecule interactions. Moreover, if allorecognition depends on low-affinity, cross-reactive recognition by T-cell receptors<sup>19</sup>, then accessory molecule interactions could make an important contribution to overall intercellular avidity.

A more stringent test of APC function is the presentation of antigen or alloantigen to freshly isolated peripheral blood mononuclear (PBM) cells. When the LDR7 transfectants were tested for the ability to stimulate alloreactive responses in HLA-mismatched PBM, they elicited no response (Fig. 3). In marked contrast, the LDR7-ICAM-1 transfectants stimulated large responses from non-HLA-DR7 (but not HLA-DR7) individuals, suggesting an important role for ICAM-1 in the induction of an alloreactive response against HLA-DR7 on these cells. But under identical conditions, the LDR2 transfectants, which express extremely high levels of HLA class II (Fig. 1), stimulated strong proliferative responses from non-HLA-DR2 PBM, without any requirement for ICAM-1 co-expression. These results provide evidence for a synergistic relationship between HLA class II and ICAM-1 in T-cell stimulation, the expression of ICAM-1 being of crucial importance under conditions of

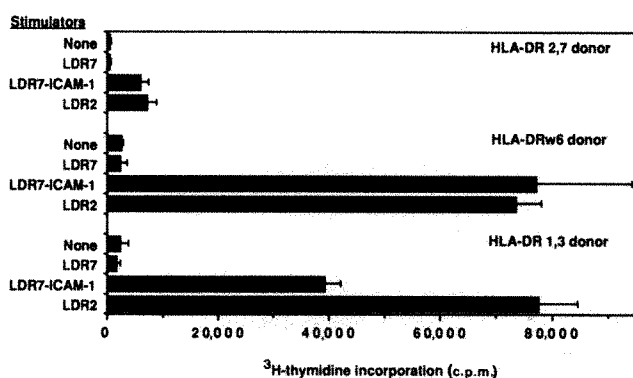
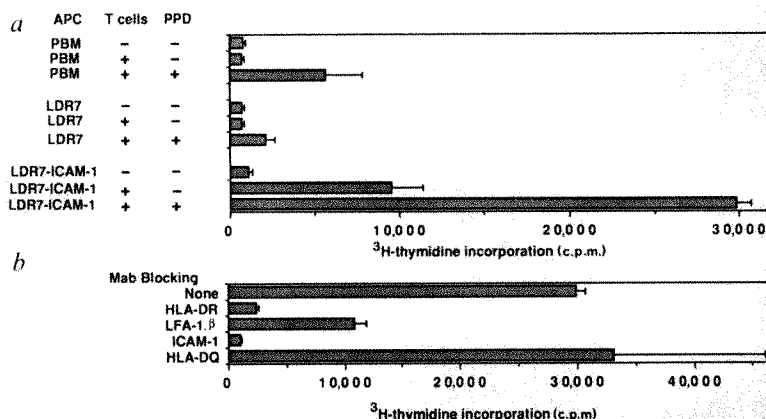


FIG. 3 Stimulation of freshly isolated peripheral blood mononuclear cells by allogeneic transfectants. Transfectants ( $5 \times 10^4$  per flat-bottom well) were treated with  $100 \mu\text{g ml}^{-1}$  mitomycin c (Sigma) at  $37^\circ$  for 1 h before co-culturing in Iscoves' modified Dulbecco's medium (Gibco Laboratories) with  $1 \times 10^5$  freshly isolated peripheral blood mononuclear cells from individuals of the indicated HLA-DR types. Responses of donors to complete HLA haplotype-mismatched B-LCL ranged  $105,000\text{--}182,000$  c.p.m. Cells were cultured for 6 d, adding  $1 \mu\text{Ci ml}^{-1}$  [ $^3\text{H}$ ] thymidine (Amersham) for the final 6 h before collecting onto filter sheets and counting in a 1205 Betaplate scintillation counter (LKB). Bars represent the mean responses ( $\pm$  s.e.) from triplicate cultures.

FIG. 4 Presentation of purified protein derivative (PPD) to freshly isolated T-cells by LDR7-ICAM-1 transfectants. *a*, Freshly isolated peripheral blood mononuclear cells from an HLA-DR2,7 individual ( $1 \times 10^5$  per well, irradiated with 40 Gy), LDR7 transfectants or LDR7-ICAM-1 transfectants (treated as described in Fig. 3 legend) were tested for the ability to present PPD of *M. tuberculosis* (Statens Serum Institute, Denmark) added at a concentration of  $25 \mu\text{g ml}^{-1}$  to autologous, nylon-wool-purified T-cells ( $1 \times 10^5$  per well). T-cells cultured with PPD in the absence of added APC gave a response of  $1,168 \pm 485$  c.p.m. Other details of culture and collecting are given in the legend to Fig. 3. Antibodies (details as in Fig. 2) were added to cultures containing purified T-cells, LDR7-ICAM-1 transfectants and PPD as in the lower part of panel *a*.



limiting HLA class II expression by the allogeneic stimulator. Similar synergistic effects between LFA-1 and class II have been reported in murine T-cell responses to antigen<sup>2</sup>. Given that the absolute level of HLA class II expressed by the LDR7 transfectants is similar to that of activated monocytes, this dramatic effect of ICAM-1 on T-cell stimulation is probably of physiological relevance.

Alloreactive T-cells may differ in their activation requirements from antigen-specific, HLA-restricted T-cells. We therefore investigated the importance of ICAM-1 expression by the APC in recall antigen responses of freshly isolated T-cells. When purified T-cells from an HLA-DR7 responder were tested for their ability to respond to a purified protein derivative of *Mycobacterium tuberculosis* (PPD), a marginal response was observed using LDR7 cells as APC (Fig. 4a), which was amplified by co-expression of ICAM-1. Antigen presentation by the LDR7-ICAM-1 transfectants was inhibited by antibodies to HLA-DR, LFA-1 $\beta$ , and ICAM-1 (Fig. 4b), confirming the HLA class II restriction and LFA-1/ICAM-1 dependence of the response. The LDR7-ICAM-1 transfectants also induced some T-cell proliferation in the absence of exogenous antigen or allogeneic HLA-DR (Fig. 4a). Indeed, L cells transfected with ICAM-1 alone delivered a small but significant mitogenic signal to purified T-cells which, although not as pronounced as the allorecognition demonstrable with LDR7-ICAM-1 transfectants, was significant compared with the response against control Ltk<sup>-</sup> cells and was specifically inhibited by mAbs against ICAM-1 or LFA-1 $\alpha$  (data not shown). Although enhancement of a small xenogeneic response to mouse class I antigens cannot be discounted, these results suggest that the effects on recognition that follow transfection of ICAM-1 may involve not only enhanced conjugate formation with T-cells, but the delivery of activation signals through the ICAM-1/LFA-1 interaction itself. Such a pathway is implied by the T-cell mitogenic effect of some LFA-1 antibodies<sup>5</sup>.

Using DNA-mediated gene transfer we have been able to define the contribution of LFA-1/ICAM-1 interactions to antigen presentation in a manner not complicated by the expression of other APC-derived accessory molecules. In the absence of high levels of HLA class II expression by the APC, ICAM-1 expression can be a decisive factor in determining whether a T-cell response occurs. This observation is relevant not only to haemopoietic APC but also to cells of non-haemopoietic origin such as fibroblasts<sup>20,21</sup>, endothelial cells, and epidermal keratinocytes<sup>22,23</sup>, which express both HLA class II and ICAM-1 following exposure to interferon- $\gamma$ , and may therefore serve as APC for self and foreign antigens<sup>24</sup>. In addition, the profound effects of ICAM-1 expression on T-cell stimulation may have direct bearing on the relationship between the lack of expression of ICAM-1 (as well as other accessory molecules) on Epstein-Barr virus Burkitt's lymphoma lines and their inability to be recognized by anti-Epstein-Barr virus cytotoxic T-cells<sup>25</sup>. □

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## Compliance of bacterial flagella measured with optical tweezers

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THE development of the gradient force optical particle trap ('optical tweezers') has made it possible to manipulate biological materials using a single beam of laser light<sup>1</sup>. Optical traps can produce forces in the microdyne range on intact cells without causing overt damage: such forces are sufficient to arrest actively swimming bacteria<sup>2</sup> and can overcome torque generated by the flagellar motor of a bacterium tethered to a glass surface by a flagellar filament. By calibrating the trapping force against Stokes' drag and measuring the twist that is sustained by this force, we determined the torsional compliance of flagella in tethered *Escherichia coli* and a motile *Streptococcus*. Flagella behaved as linear torsion springs for roughly half a revolution, but became much more rigid when turned beyond this point in either direction.

Many motile bacteria are propelled by flagellar filaments, each of which is turned at its base by a reversible rotary motor driven by a transmembrane proton gradient<sup>3-5</sup>. In *E. coli*, the filament is a long helical polymer of a protein, flagellin, of molecular weight about 55,000. The filament is joined to the motor by means of a short hook (~80 nm long), a polymer of another protein, that is believed to serve as a flexible coupling<sup>3,5</sup>. The filament, hook and motor (or basal structure) constitute the flagellum. Wild-type filaments are polymorphic: left- and right-handed forms have been described with a variety of helical pitches, depending on pH, ionic strength and torsional load<sup>5</sup>. Under normal physiological conditions, they assume a left-handed configuration, with wavelength ~2.5  $\mu$ m. When the motors turn anticlockwise (as viewed looking down the filament at the cell), the filaments move together to push the cell forward. When the motors turn clockwise, the filament bundle flies apart and the cell tumbles. Polymorphic transitions have been observed during tumbling which convert the filaments to right-handed forms with roughly half the wavelength<sup>6</sup>. When tethered to a surface by a single filament, a cell spins alternately clockwise and anticlockwise at nearly the same angular speed, changing direction abruptly and at random<sup>7,8</sup>, some cells occasionally pause<sup>9</sup>. At room temperature, filaments in bundles spin at about 100 Hz, whereas tethered cell bodies spin at about 10 Hz. The motor exerts its maximum torque at stall, dropping monotonically with increased speed<sup>10</sup>.

Here we used cells whose motors had been paralysed by mutation, or de-energized by starvation or treatment with agents that dissipate the transmembrane proton gradient. These cells were tethered and spun by an external torque that was applied with an optical trap (Fig. 1). The central component is an infrared laser (wavelength 1,064 nm) whose beam is brought to a diffraction-limited focus by an objective of high numerical aperture. Transparent refractile materials such as bacteria experience forces that arise from induced dipole interactions



with the light gradient, which tend to pull them into a trapping zone located just beyond the focus. The dimensions of the zone are comparable with the wavelength. Beam-steering optics allow the trap to be positioned manually in  $x$ ,  $y$ , and  $z$  dimensions and swept electronically clockwise or anticlockwise in a circle in the  $x$ - $y$  (specimen) plane. The laser is equipped with an attenuator to adjust the force and with a shutter to gate the trap.

At a low Reynold's number ( $\sim 10^{-5}$  in the case of *E. coli*), the externally applied torque is balanced by torques arising from viscous drag on the cell body, torsion in the tether and thermal motion

$$f_{\theta}\Omega(t) + k_{\theta}\theta(t) + L(t) + N(t) = 0 \quad (1)$$

where  $f_{\theta}$  is the rotational drag coefficient of the cell body,  $\Omega(t)$  is its angular velocity,  $k_{\theta}$  is the torsional spring constant of the tether,  $\theta(t)$  is its twist,  $L(t)$  is the Langevin torque arising from random bombardment by molecules in the medium,  $N(t)$  is the applied torque and  $t$  is the time. Three kinds of behaviour can be distinguished, depending on the degree to which the motor resists rotation. (1) The motor turns freely, that is, it swivels about its tether and  $k_{\theta}\theta$  vanishes. (2) The motor locks up partially, that is, it fails to turn until the torque arising from twist in the tether reaches a finite value, whereupon it slips;  $k_{\theta}\theta$  has some upper bound. (3) The motor locks up entirely, that is, the cell body becomes clamped to the end of the tether, in which case  $\Omega(t) = d\theta/dt$ . Measurements on partially or fully locked cells enable  $k_{\theta}$  to be determined. Note that for small angular deviations, the behaviour of partially or fully locked cells is the same.

*E. coli* carrying mutations in *motA* or *motB*, the genes implicated in torque production, have flagella that fail to rotate<sup>11-13</sup>. We found that tethered cells in which either or both genes had been deleted diffused freely, as judged by videotape recordings and time-exposure photomicrographs; the time for a random excursion through  $2\pi$  radians was typically several minutes, consistent with free Brownian rotation. When these cells were

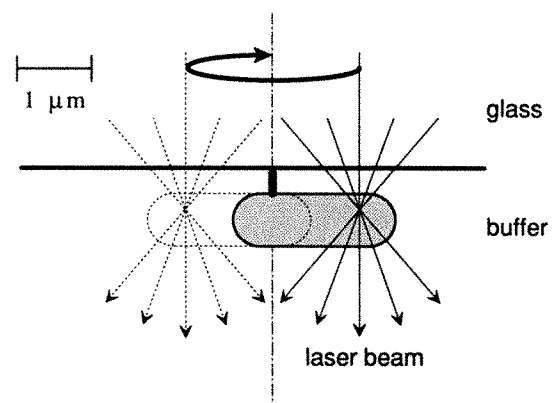
spun through several revolutions by the optical trap and then abruptly released by shuttering the laser, they stopped in place. The external torque required to rotate cells carrying the double deletion  $\Delta(motA-motB)$  was proportional to the angular velocity (measured from  $0.4 \times 10^{-11}$  dyne-cm at 0.5 Hz to  $2.6 \times 10^{-11}$  dyne-cm at 3.0 Hz); least-squares fits passed through the origin.

Measurements of this kind were quantitated by calibrating the trap against Stokes' drag. A free cell was captured by the trap; this orientated its long axis parallel to the beam. The light level was fixed with the attenuator and the cell was moved along a circular trajectory ( $\sim 4 \mu\text{m}$  in diameter). This path was retraced at increasing angular velocity until the transverse drag on the cell was just sufficient to pull it from the trap, that is, until the trapping force was balanced by Stokes' drag. This 'critical angular velocity' was recorded over the range of attenuator settings and a calibration curve constructed. As anticipated, this velocity (and hence the force) was strictly proportional to light level. The drag force was computed from the critical angular velocity, circumference of the trajectory, viscosity, and the transverse linear drag coefficient for that cell. To compute the drag coefficient, the cell's shape was approximated by a cylinder with hemispherical endcaps<sup>14</sup>; measurements of its dimensions were made from recordings using a computer-based video measurement system under conditions of negligible blooming<sup>15</sup>. Torques applied to tethered cells were computed from the product of the trapping force and the trap eccentricity (distance from centre of trap to centre of rotation). A correction factor equal to the aspect ratio of the cell was applied to the calibration to compensate for the optical path through the cell being shorter when its long axis was perpendicular to the laser beam. This correction gave excellent agreement between the measured slopes of the torque-velocity curves for  $\Delta(motA-motB)$  cells and their computed rotational drag coefficients<sup>14</sup>.

Wild-type cells normally spin when tethered, but their motors

FIG. 1 Sketch of a bacterial cell being rotated about its tether by the optical trap. The axis of rotation of the trap (dashed line) was aligned with the tether (short thick line). The sense of rotation is shown by the circular arrow. Two views of the cell are displayed, at  $\theta = 0^\circ$  (solid lines) and  $\theta = 180^\circ$  (dotted lines). The laser beam enters from above, through the coverslip, as shown by the crossed rays. The microscope illumination (not shown) enters from below.

**METHODS.** Cells of *E. coli* strains RP437 (wild type for motility and chemotaxis), RP6665 ( $\Delta(motA-motB)$ ), RP421 (*motB*<sup>580</sup>) and RP6894 ( $\Delta(motA-motB)$ ) containing plasmid SYC62 encoding wild-type *motB* (gifts from S. Parkinson) were grown at 35 °C in tryptone broth, washed twice in buffer, shorn, washed twice again, and tethered as described<sup>24</sup>, except that the buffer was 0.01 M potassium phosphate, pH 7.0, made 0.067 M in NaCl and  $10^{-4}$  M in EDTA, and cover slips were cleaned with acetone followed by saturated KOH in ethanol. Cells of *Streptococcus* strain V4051 were grown at 35 °C in 1%  $\text{K}_2\text{HPO}_4$ , 1% tryptone, 0.5% yeast extract, 1% glucose medium, washed twice in buffer, shorn, washed twice again, and tethered as described previously<sup>25</sup>, except that the buffer used was 0.01 M TES, 0.01 M MES (pH 8.5), 0.2 M KCl,  $10^{-4}$  M EDTA; cover slips were cleaned as above. The cover slips were mounted in a small flow cell<sup>26</sup>. Experiments were at room temperature. *E. coli* were de-energized by flowing in buffer containing 2,4-dinitrophenol (DNP, 3 or 10 mM) for several minutes. *Streptococci* were de-energized by starvation for  $\geq 30$  min in buffer, or by treatment with buffer containing DNP (3 mM) or trifluoromethoxycarbonyl-cyanide phenylhydrazine (FCCP,  $10 \mu\text{g ml}^{-1}$ ). To suppress possible photodynamic effects with DNP, a 1 cm-thick filter containing DNP ( $\sim 10$  mM) was interposed between the microscope lamp and the specimen. The optical trap comprised a Nd:YAG diode-pumped microlaser (Amoco Laser Co., ALC-1064, selected for 95 mW power output), a  $3\times$  beam expander, a relay lens (CVI Laser, laser aplanat) held by a micropositioner, an infrared/visible dichroic mirror (CVI Laser) mounted in the epi-head of a research microscope (Zeiss, Universal), and a phase-contrast objective of high numerical aperture (Nikon, 60 $\times$  Planapo, DM series, 1.4 NA). The beam diameter was adjusted to fill the rear pupil of the objective. Light intensity was varied with a dual-wedge compensating attenuator (Newport, 925B) and gated by an electronic shutter (Uniblitz, 26L). Coarse  $x$ - $y$  beam steering and  $z$ -focusing were achieved by adjusting the position of the relay lens with micrometer



screws. The relay lens was mounted at the end of a rigid cantilever; fine  $x$ - $y$  steering was achieved by energizing coils that wrapped around this cantilever and extended through fields generated by permanent magnets. The steering system had a resonant frequency of 33 Hz and was critically damped with a dashpot filled with a halocarbon oil. The coils were driven by a quadrature oscillator that produced circular displacement of the lens: the direction, centre position, speed and amplitude of the circle were adjustable. The trap position was monitored by a charge-coupled device camera (Pulnix, TM-840N) that picked up the infrared reflection of the beam spot off the cover slip; the same camera was used to videotape all experiments. In control experiments designed to assess possible damage caused by high laser light levels, *Streptococcus* was tethered in growth medium and arrested with the beam at full power. The beam was gated off for a few seconds every minute to release the cell and allow it to spin. No reduction in motility was observed over a period of hours; indeed, the cell elongated and eventually divided. *E. coli* was far less resistant to light; cells lost motility after several minutes' exposure. Therefore, we used minimal light levels and kept the laser shuttered at all times when measurements were not being made.

can be arrested by treatment with protonophores such as 2,4-dinitrophenol (DNP) and trifluoromethoxycarbonyl-cyanide phenylhydrazone (FCCP). In the case of *Streptococcus*, motors can also be halted by starvation<sup>4</sup>. These treatments produced a range of phenotypes. In contrast with previous reports<sup>4,16</sup>, we found that many of these cells were able to diffuse freely. In some cases this motion was hindered at certain fixed angles, probably as a result of interactions with the tethering surface. Other cells locked up, either partially, so that the cell could still be driven through many revolutions with the light trap, or entirely, so that it could not. When a partially locked cell was spun through several revolutions and then abruptly released, the cell body rebounded elastically through a fraction of a revolution (typically 60°). For example, in an experiment with starved *Streptococcus*, a cell was spun either clockwise or anticlockwise at 1.5 Hz for several seconds and then released at a random angle. The rebound angle was distributed normally with a mean and s.d. of  $61^\circ \pm 27^\circ$  ( $n=108$ ). The amplitude of the recoil seemed to be independent of the direction of rotation and diminished by only 15% when the initial angular velocity was halved. There was no obvious spatial periodicity to the angular positions of the cell after rebounding. When we measured the external torques that were required to turn partially locked, DNP-treated cells of *E. coli* as a function of angular velocity, we obtained straight lines which were displaced upwards from the origin by  $\sim 2 \times 10^{-12}$  dyne-cm. This corresponds to an additional torque required to overcome the barrier caused by the partial lock-up.

The lock-up behaviour of protonophore-treated *E. coli*, or of starved or protonophore-treated *Streptococcus*, implies the presence of an energy barrier to rotation which is larger than  $kT$ , where  $k$  is Boltzmann's constant and  $T$  is the absolute temperature. A typical record of jitter angle for *Streptococcus* is displayed in the inset to Fig. 2. By the equipartition theorem,  $kT/2 = k_\theta \langle \theta^2 \rangle / 2$ , permitting an estimate of  $k_\theta$  directly from the mean-square angle<sup>16</sup>. For this cell, we obtained  $k_\theta = 1.18 \times 10^{-12}$  dyne-cm rad<sup>-1</sup>. The autocorrelation of the angle,  $I(\tau)$ , is obtained by solving equation (1) with  $N(t)=0$  and  $\Omega(t) = d\theta/dt$ , giving  $I(\tau) = I_0 \exp(-|\alpha\tau|)$ , where  $\tau$  is the lag

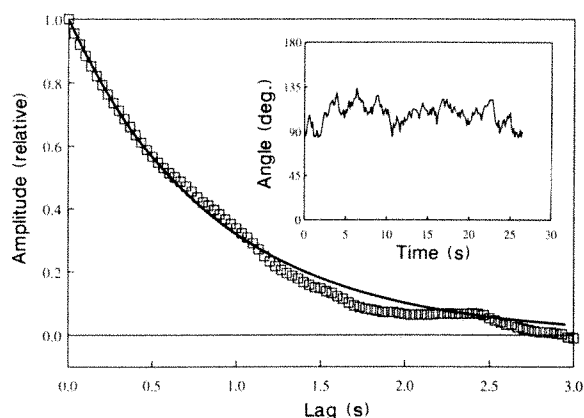


FIG. 2 Noise analysis of tethered *Streptococcus*. The graph shows the normalized autocorrelation function (squares) of angular jitter due to Brownian rotation of a partially locked *Streptococcus* cell treated with FCCP. The data were fitted by an exponential of time constant 0.875 s, solid line. Inset: The data from which the autocorrelation was computed, showing the angular position of this cell for 26.4 s. The root-mean square amplitude of the angular distribution was  $10.7^\circ$ .

**METHODS.** The autocorrelation as computed by standard fast-Fourier-transform technique<sup>27</sup> from an 800-point record of the angular position, with points digitized at every video-frame interval (1/30 s). Angles were measured with a computerized 'video protractor' coupled to an incremental shaft encoder (Vernitec, VEOL-23). Measurement error, determined from recordings of stuck cells, was  $\pm 4^\circ$ . Mean-square angle is a biased estimator, because zero-mean noise adds as a square, so values were corrected by subtracting the squared measurement error.

time and  $\alpha = k_\theta / f_\theta$  (ref. 17). The experimental data are fitted by an exponential of time constant  $\alpha^{-1} = 0.875$  s (Fig. 2). The rotational drag coefficient,  $f_\theta$ , was estimated from the cell's geometry and the viscosity<sup>13</sup>, giving  $k_\theta = \alpha f_\theta = \alpha f_\theta = 1.21 \times 10^{-12}$  dyne-cm rad<sup>-1</sup>.

A more direct measurement of  $k_\theta$  was made from the kinetics of the rebound that occurred when a partially locked cell was spun and released (Fig. 3). In this relaxation experiment,  $N(t)$  is reduced from a fixed value to zero, and  $\theta(t)$  decays as  $\exp(-\alpha t)$  to zero. The upper curves display the angle as a function of time after release, using the same cell as shown in Fig. 2. The angle relaxed exponentially, as required by equation (1), with time constant  $\alpha^{-1} = 0.745$ , giving  $k_\theta = \alpha f_\theta = 1.42 \times 10^{-12}$  dyne-cm rad<sup>-1</sup>. This value is slightly larger than that obtained by noise analysis, in which a small amount of drift in the steady-state angular position would exaggerate  $\langle \theta^2 \rangle$ , leading to an underestimate of  $k_\theta$ . The lower curves of Fig. 3 show analogous data for *E. coli*, with a time constant of  $\alpha^{-1} = 0.143$  s, nearly five times faster, and giving  $k_\theta = 4.03 \times 10^{-12}$  dyne-cm rad<sup>-1</sup>. Cells of *E. coli* are smaller and their flagella appear to be more than twice as stiff, a property reflected in the small root-mean-square jitter observed in tethered *E. coli* ( $\sim 6^\circ$ ), compared with *Streptococcus* ( $\sim 11^\circ$ ).

A motor driving a tethered cell of *Streptococcus* generates a torque of  $\sim 3 \times 10^{-11}$  dyne-cm<sup>10</sup>. Were the flagellum to behave as an ideal spring throughout its full range of movement with  $k_\theta \approx 1.3 \times 10^{-12}$  dyne-cm rad<sup>-1</sup> (the value determined above), it would twist nearly four complete turns when subjected to this torque. Were the motor to reverse suddenly, the cell body would not follow suit until the flagellum had unwound four turns, and it would not reach top speed until the flagellum had wound up four turns in the opposite direction. Even if the motor were run at its maximum speed throughout this manoeuvre (100 Hz at room temperature<sup>10</sup>), the reversal would take roughly 0.1 s. Reversals are much more abrupt than this ( $< 0.01$  s for *E. coli*<sup>16</sup> and  $< 0.03$  s for *Streptococcus*; S.M.B., D.F.B. and H.C.B., unpublished data).

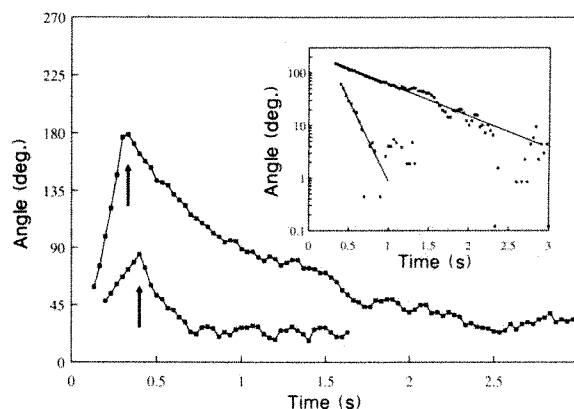


FIG. 3 Relaxation experiments with partially locked bacteria. The upper curve in the main plot shows the angle of the *Streptococcus* cell of Fig. 2 as a function of time, digitized at each video-frame interval. The cell was driven anticlockwise by the light trap and released at the time indicated by the arrow. The cell rebounded clockwise, relaxing to equilibrium. The lower curve shows a similar experiment with a wild-type cell of *E. coli* treated with 10 mM DNP. Inset: Semilog plots of these data from the time of the arrows, after subtraction of the baseline angle, and linefits (solid lines) showing the exponential character of the relaxation. Time constants for decay and computed rotational drag coefficients: 0.745 s,  $1.06 \times 10^{-12}$  dyne-cm rad<sup>-1</sup> s<sup>-1</sup> and 0.143 s,  $5.76 \times 10^{-13}$  dyne-cm rad<sup>-1</sup> s<sup>-1</sup> for *Streptococcus* and *E. coli* respectively.

**METHODS.** The attenuator was set to produce negligible trapping force, the angular speed was set to 0.5 Hz, and the trap was aligned with a tethered cell, as illustrated in Fig. 1. The beam was then shuttered and the attenuator reduced to a setting that gave strong trapping. The shutter was opened and the cell spun at a fixed speed between 0.5 and 2 Hz for a few seconds, at which point the shutter was closed and the angular relaxation recorded.

We resolved this paradox by determining compliance over a large angular range. Figure 4 shows the torsion angle as a function of torque for cells whose motors appeared fully locked. In this domain, for sufficiently slow rotation,  $N \approx -k_\theta \theta$ . The compliance (slope) shows linear elastic behaviour through some  $100^\circ$  of twist (with a value of  $k_\theta$  similar to that found above), and then it becomes more than an order of magnitude stiffer. Because of the nonlinearity, a tethered cell needs only to twist its flagellum about half a turn before it becomes rigid; therefore, a reversal could be completed within one turn, namely in  $\leq 0.01$  s. In a parallel experiment, a wild-type cell was arrested by gentle fixation with glutaraldehyde. The compliance was symmetric for both clockwise and anticlockwise rotations (Fig. 4, inset). The smaller asymptotic angle observed for this cell might reflect the difference in tether lengths, which can vary from cell to cell.

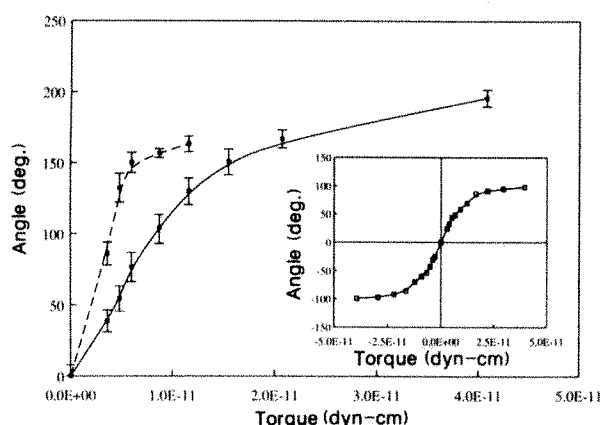


FIG. 4 Compliance curves for locked bacteria. The graph shows the angular displacement of fully locked cells as a function of the torque applied by the optical tweezers. Dashed line, data from a cell of *Streptococcus* treated with 10 mM DNP. Value for  $k_\theta$  at small angles  $\sim 2 \times 10^{-12}$  dyne-cm rad $^{-1}$ . Solid line, data from a cell of *E. coli* treated with 10 mM DNP. Value for  $k_\theta$  at small angles  $\sim 5 \times 10^{-12}$  dyne-cm rad $^{-1}$ . Error bars show the standard deviation for ten or more determinations of angle. Inset: Compliance curve for a cell of *E. coli* fixed with 0.1% glutaraldehyde in buffer, showing symmetry for clockwise and anticlockwise rotation.

**METHODS.** Cells which were apparently locked at a fixed angle were found by trial and error after treatment with 10 mM DNP. The trap was aligned as illustrated in Fig. 1, its speed was fixed at 0.25 Hz, and the eccentricity adjusted to maximize the torque. Then the beam was shuttered. The attenuator was reduced in a series of steps and the shutter opened for a short time at each level. As the trap encountered the cell body, it entrained it, turning it through part of a revolution until the trapping torque just balanced the elastic reaction of the tether. At this point, the cell escaped from the trap and rebounded to its original angle. This process was repeated at least ten times for each setting of the attenuator. Single-frame analysis with the video protractor provided the maximum angle attained for each trial. The force calibration curve and the trap eccentricity were used to convert the attenuator settings to torque. For most cells of *Streptococcus* (tethered without antibody), the highest values of torque would twist the cell off the tethering surface, terminating the experiment; this did not occur for cells of *E. coli* (tethered with antibody). In one instance involving *Streptococcus*, the cell remained well tethered, but the cell body suddenly stopped rebounding, remaining instead where it was when the beam was shuttered. Either the mechanism responsible for the lock-up was overcome or a component of the motor was broken. The torsion constant of a uniform hollow tube is related to the torsional rigidity,  $\mu$ , by  $k_\theta = \pi\mu(R^4 - r^4)/2L$ , where  $r$  and  $R$  are the inner and outer radii respectively, and  $L$  is the length<sup>28</sup>. Using  $R = 70$  Å and  $r = 30$  Å, corresponding to the radii of maximum (rod) density and of the inner hole, respectively, for filaments of *Salmonella typhimurium*<sup>20</sup>, and our values of  $L = 0.2$  μm and  $k_\theta = 4 \times 10^{-12}$  dyne-cm rad $^{-1}$ , we obtain  $\mu = 2 \times 10^9$  dyne cm $^{-2}$ , which is 3–4 orders of magnitude lower than an estimate<sup>19</sup> ( $10^{11}$ – $10^{12}$  dyne cm $^{-2}$ ) derived from filaments of *S. typhimurium* that had been polymerized *in vitro*, but comparable to an estimate<sup>16</sup> ( $\sim 10^9$  dyne cm $^{-2}$ ) that was based on noise analysis of tethered *E. coli*. An explanation for this discrepancy could be that there is an additional compliance in flagella that is not present in filaments (see text and ref. 19). We note that the torsion constant attained by *E. coli* flagella at large angles is at least  $1 \times 10^{-10}$  dyne-cm rad $^{-1}$ , implying that  $\mu \geq 5 \times 10^9$  dyne cm $^{-2}$ .

These lengths range from 0.1–0.5 μm, as estimated from the lateral displacements of cells that occur during normal reversals, or when cells are moved sideways with the optical trap. We note that both curves for *E. coli* in Fig. 4 are nearly superimposable when normalized to the maximum angle.

What is the explanation for the shape of the compliance curve? One possibility is that flagellar compliance consists of two distinct components: a 'soft' initial phase resulting from twisting of the proximal hook, and a 'hard' terminal phase dominated by the filament once the hook has reached some elastic limit. Published estimates of the flexural and torsional rigidity of isolated filaments<sup>18,19</sup> indicate that they are far stiffer than our values of  $k_\theta$  would imply (compare Fig. 4). It is also possible that the compliance is entirely dominated by the filament itself and that it is intrinsically nonlinear. A 0.2 μm-long tether carries roughly 80 turns of the lowest-order helical lattice, which has a pitch close to 26 Å and an outer diameter close to 200 Å (ref. 20). Twisting such a filament through one-half revolution would impose more than  $2^\circ$  of superhelical twist (shear) per turn, requiring a substantial accommodation of the structure. These possibilities could be distinguished by measuring the elastic properties of polyhook strains, which produce abnormally long hooks<sup>21</sup>, and/or by making compliance measurements over a wide range of filament lengths. We do not believe that the compliance is due to, say, elasticity of force generators which link the rotor to the cell wall, because DNP treatment also induced partial lock-up of  $\Delta(motA-motB)$  cells. In relaxation experiments, these cells rebounded like wild-type cells. We did not see any abrupt changes in compliance which might be attributable to polymorphic transitions in the filament, even though the torques exceeded those experienced by flagella in tumbling cells, where such interconversions have been observed<sup>6</sup>. It is possible that interconversions did occur but remained undetected because of the shortness of the tethers, which are only a fraction of the normal flagellar wavelength. It is also possible that anti-filament antibody bound to the tether or tight association with the surface at the filament's distal tip prevented such cooperative transitions.

This elastic nonlinearity could be useful in motility. The filaments need to be stiff to function efficiently as propellers. But to form a coherent bundle, flagella emerging from distinct motors distributed over all parts of the cell body must coalesce, with attendant distortion of their helical geometry<sup>22</sup>. Early events in bundle formation may take advantage of a relatively soft (hook-based?) compliance to stabilize the coordinated bundle. We note that the torque produced by motors in actively swimming *Streptococcus* ( $\sim 1 \times 10^{-11}$  dyne cm $^{10}$ ) is just sufficient to wind flagella beyond the linear compliance domain.

Optical tweezers provide a novel means of measuring the elastic properties of individual bacterial flagella, means that are hard to duplicate with conventional techniques, for example, those using thin glass needles<sup>23</sup>. We anticipate that trapping technology will be applicable to the manipulation of a wide range of macromolecular assemblies. □

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## Functional significance of the Kunitz-type inhibitory domains of lipoprotein-associated coagulation inhibitor

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**BLOOD** coagulation can be initiated when factor VII or VIIa, a plasma protease, binds to its essential cofactor, tissue factor (TF), and proteolytically activates factors IX and X<sup>1,2</sup>, triggering a cascade of events which eventually leads to the formation of thrombin and a fibrin clot. Plasma contains a lipoprotein-associated coagulation inhibitor (LACI) which inhibits activated factor X (Xa) directly and, in a Xa-dependent way, inhibits VII(a)/TF

activity, presumably by forming a quaternary Xa/LACI/VII(a)/TF complex<sup>3-5</sup>. Sequence analysis of complementary DNA clones has shown that LACI contains three tandemly repeated Kunitz-type serine protease inhibitory domains<sup>6,7</sup>. To investigate the relationship between these Kunitz structures and LACI function, we have used site-directed mutagenesis to produce altered forms of LACI in which the residue at the active-site cleft of each Kunitz domain has been individually changed. The second Kunitz domain is required for efficient binding and inhibition of Xa, and both Kunitz domains 1 and 2 are required for the inhibition of VIIa/TF activity; but alteration of the active-site residue of the third Kunitz domain has no significant effect on either function. We propose that in the putative inhibitory complex, Kunitz domain 1 is bound to the active site of VII(a)/TF and that Kunitz domain 2 is bound to Xa's active site.

Bovine pancreatic trypsin inhibitor (BPTI, aprotinin) is the best characterized Kunitz-type inhibitor and alteration of the residue in the P1 position<sup>8</sup> of the active-site cleft of BPTI profoundly alters its inhibitory activity<sup>9</sup>. By sequence homology alignment with BPTI, the P1 position for each of LACI's Kunitz domains was identified (ref. 6; Fig. 1). We used site-directed mutagenesis to prepare LACI mutants in which this active-site-cleft residue in each Kunitz domain has been individually changed. Thus, the mutants were designed to maximize the effect on each domain's function while minimizing the possible alteration in the protein structure. These modified LACI DNAs were introduced into the bovine papilloma virus vector pMON1123 and cotransfected with pSV2neo into mouse C127 fibroblasts.

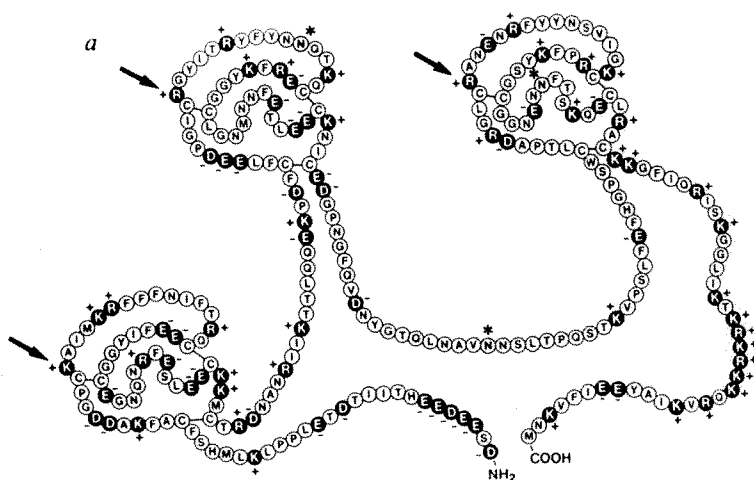
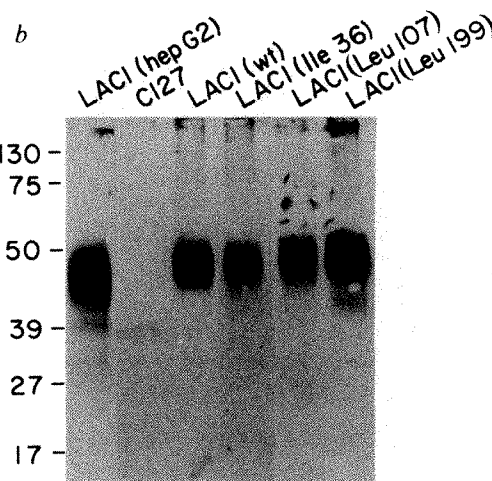


FIG. 1 *a*, Schematic diagram of LACI's predicted secondary structure showing the sulphhydryl bonding for the three Kunitz domains. The Kunitz domains are numbered from the one closest to the N terminus. The arrows indicate the location of the presumed P1 residues of the active-site clefts for the Kunitz domains<sup>8,9</sup>. The charges of the amino-acid side chains are indicated (histidine side chains are considered as uncharged). Asterisks show the potential sites for N-linked glycosylation. *b*, Western blot showing the expression of the recombinant wild-type and mutant LACIs. Serum-free conditioned media (100  $\mu$ l) from the indicated cells and 20 ng purified LACI(hepG2) were run on a 15% polyacrylamide gel, transferred to nitrocellulose, probed with mouse polyclonal anti-LACI antibodies and colourimetrically developed<sup>7,10</sup>. Relative molecular masses are shown on the left in thousands.



**METHODS.** A modified LACI cDNA insert engineered into a bovine papilloma virus vector for expression in C127 fibroblasts<sup>7</sup> was ligated into M13mp18 and site-directed mutagenesis<sup>11</sup> was performed as follows (the numbering of nucleotides and amino acids is according to the 4.0 kilobase (kb) LACI cDNA sequence<sup>7</sup>. LACI(Ile 36): A  $\rightarrow$  T at 572 which changes Lys 36 to Ile. LACI(Leu 107) G  $\rightarrow$  T at 785 which changes Arg 107 to Leu. LACI(Leu 199): G  $\rightarrow$  T at 1,061 which changes Arg 199 to Leu. Sequences of the mutant molecules were confirmed by the dideoxy chain-termination procedure<sup>12</sup> and cloned into the bovine papilloma virus expression vector pMON1123 (ref. 7). Each of these expression vectors plus pSV2neo were cotransfected into mouse C127 fibroblasts using calcium phosphate precipitation<sup>3</sup> and G418-resistant clones were screened for expression of LACI. LACI(hepG2) was purified as previously described<sup>14</sup>.

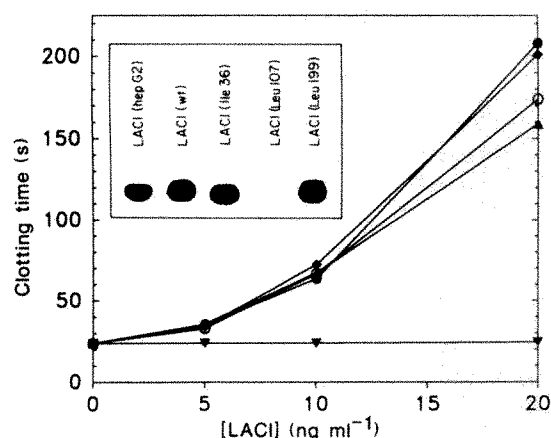


FIG. 2 Binding and inhibition of factor Xa by wild-type and mutant LACIs. Factor Xa inhibitory activities were determined as previously described<sup>3</sup> using 25 ng ml<sup>-1</sup> (final concentration) bovine factor Xa. Samples are: LACI(hepG2) (○); LACI(wt) (●); LACI(Ile 36) (▲); LACI(Leu 107) (▼); LACI(Leu 199) (◆). Inset, <sup>125</sup>I-labelled Xa blot analysis of LACI mutants. Samples are designated in the same way as in Fig. 1.

**METHODS.** LACIs were precipitated from transfected cell clones' serum-free conditioned media using CdCl<sub>2</sub>, resuspended in 0.25 M EDTA, pH 9.0, clarified and dialysed into 0.1 M NaCl, 0.05 M Tris-HCl, pH 7.5 (TS buffer)<sup>14</sup>. Recombinant LACIs were purified using a mouse monoclonal anti-LACI-affigel 10 column. Dialysed CdCl<sub>2</sub>-concentrated LACI samples were applied to the column in TS, the column was washed with several volumes of TS and LACI was eluted with 2 M NaSCN. After the addition of BSA as carrier, concentration and dialyses into TS, the LACI concentration for each sample was determined in a particle-concentration immunofluorescence assay<sup>15</sup> using two non-competitive anti-LACI monoclonal antibodies. For the ligand blot, 50 ng of each sample was electrophoretically fractionated by SDS-PAGE, transferred to nitrocellulose, and then probed with <sup>125</sup>I-labelled Xa (refs 7, 10). Western blot analysis using rabbit polyclonal anti-LACI antibodies was performed on a duplicate blot to confirm that equivalent amounts of LACI were present in each sample (data not shown).

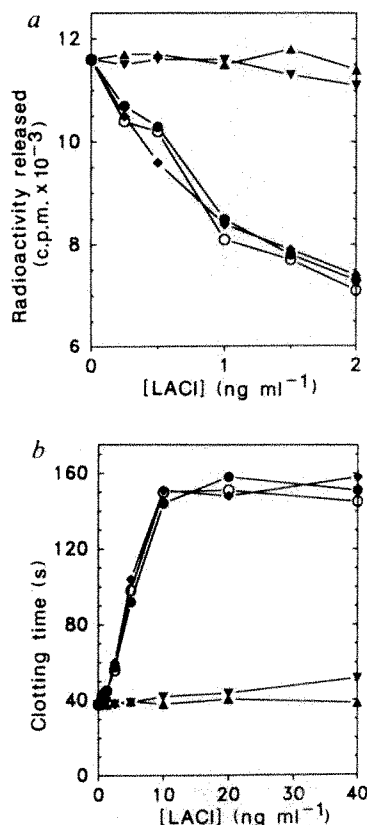


FIG. 3 VII(a)/TF inhibitory activities of the wild-type and mutant LACIs measured by a [<sup>3</sup>H] IX activation peptide release assay (a) and a three-stage clotting assay (b). Both assays were performed as previously described<sup>3,7</sup> with LACI(hepG2) (○); LACI(wt) (●); LACI(Ile 36) (▲); LACI(Leu 107) (▼); LACI(Leu 199) (◆).

Individual clones expressing human LACI messenger RNA (data not shown) and LACI protein were identified (Fig. 1) and recombinant LACIs were isolated from the clones' conditioned media using monoclonal anti-LACI affinity chromatography.

LACI isolated from the conditioned media of a human hepatoma cell line (hepG2), as well as that isolated from plasma, bind <sup>125</sup>I-labelled factor Xa on ligand blots and inhibit the enzymatic activity of factor Xa (refs 3 and 10; W.F.N. *et al.*, manuscript submitted). We assessed the ability of the modified LACI molecules to bind to and inhibit factor Xa. LACI(Ile 36) and LACI(Leu 199) with an altered active-site residue for Kunitz domains 1 and 3, respectively, both bound <sup>125</sup>I-labelled Xa on ligand blots and inhibited factor Xa to the same degree as recombinant wild-type LACI LACI(wt) or LACI(hepG2). But LACI(Leu 107), which contains an altered active-site residue in the second Kunitz domain, was not recognized by <sup>125</sup>I-labelled Xa, nor did it inhibit factor Xa (Fig. 2). These results indicate that the second Kunitz domain is responsible for the binding to, and inhibition of, factor Xa by LACI.

The ability of the altered LACI molecules to inhibit VII(a)/TF activity in the presence of Xa was determined using two separate assays. In the first, VII(a)/TF activity was determined by the release of the activation peptide from its substrate factor IX (ref. 7). LACI(Leu 199), which contains an altered Kunitz domain 3, inhibited the VII(a)/TF activity to the same degree as LACI(wt) and LACI(hepG2) (Fig. 3). Modification of either the first Kunitz domain, LACI(Ile 36), or the second Kunitz domain, LACI(Leu 107), did result, however, in the loss of VII(a)/TF inhibitory activity.

Similar results were obtained for the mutant proteins in a three-stage clotting assay which is dependent on VII(a)/TF activation of its other substrate, factor X. In this assay, inhibition of VII(a)/TF activity by LACI depends on the presence of factor X (which is converted to Xa) in the first stage of the assay<sup>3</sup>. Inhibition of VII(a)/TF activity in the first stage causes less factor X to be activated in the second stage, so clotting takes a

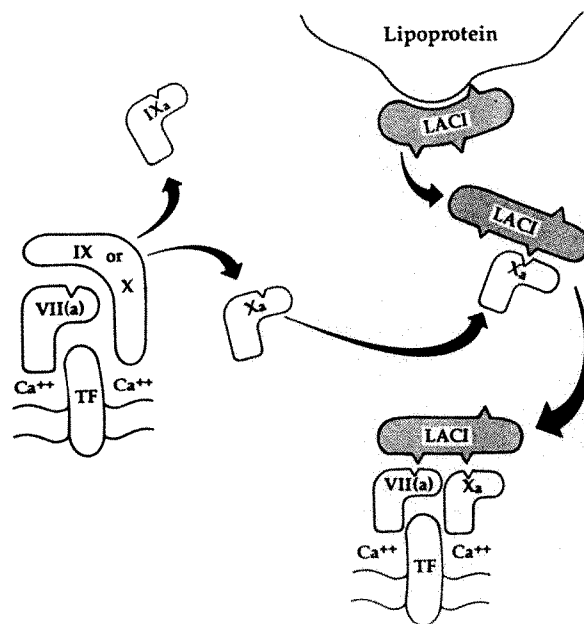


FIG. 4 Schematic diagram of the proposed mechanism for the inhibition of factor Xa and the VII(a)/TF complex by LACI. VII(a) denotes either VII or VIIa; the indentations represent the active sites for VII(a) and Xa; and the protrusions represent the three Kunitz domains of LACI. In the Xa/LACI complex, the active site of Xa is bound to the second Kunitz domain of LACI. In the final quaternary Xa/LACI/VII(a)/TF complex Xa is bound at its active site to LACI's second Kunitz domain and VII(a) is bound at its active site to the first Kunitz domain of LACI. The mechanism of the association of LACI with lipoproteins is not known.

longer time. Again, mutation of the active-site residue in either the first or second Kunitz domain abrogated LACI's ability to inhibit VII(a)/TF activity effectively, whereas mutation of the third Kunitz domain had no effect on activity. At high concentrations of LACI(Leu 107) a slight but reproducible inhibition was observed (Fig. 3). This low level of inhibition was also dependent on the presence of Xa in the first stage of the assay (data not shown).

Our current working hypothesis for the mechanism by which LACI inhibits VII(a)/TF activity is schematically shown in Fig. 4. When plasma is exposed to TF, VII(a) binds to TF and activates factors IX and X. Some of the Xa generated becomes bound by its active site to LACI's second Kunitz domain and is inhibited. This Xa/LACI complex then binds to, and inhibits, the VII(a)/TF complex, presumably by forming a Xa/LACI/VII(a)/TF quaternary complex. Alternatively, LACI may bind to a preformed VII(a)/TF/Xa complex<sup>3</sup>. We have previously shown that the N-terminal domain of Xa, containing its  $\gamma$ -carboxyglutamic acids which are required for  $\text{Ca}^{2+}$  binding, is necessary for inhibition of VII(a)/TF activity by the LACI/Xa complex<sup>3</sup>. The results presented here indicate that the second Kunitz domain is necessary for the formation of the Xa/LACI complex; this, and also the first Kunitz domain, are required for VII(a)/TF inhibition. The effect of domain 1 is presumably

mediated through its binding to the active site of VII(a). The native P1, residue of LACI's third Kunitz domain, however, does not seem to be required for these functions. It is conceivable that changing Arg 199 to Leu was not sufficient to affect functional activity of the third domain, or that a portion of the domain unrelated to its active-site cleft is required for function, but we believe that both these possibilities are unlikely.  $\square$

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## Conformational dynamics of individual DNA molecules during gel electrophoresis

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GEL electrophoresis is widely used in molecular biology to separate DNA molecules according to their sizes. The physical basis of this size separation is, however, poorly understood. Here we report observations of individual, fluorescently stained DNA molecules as they migrate during various kinds of gel electrophoresis. Their movement, under the influence of either a steady electric field or a pulsed-field, is characterized by cycles of elongation and contraction. Initially relaxed coils of DNA lengthen into 'hook-shaped' configurations which temporarily 'hang-up' on obstacles in the gel matrix before sliding off, contracting and entering another cycle. The effects of a new electrophoresis technique, termed 'pulse-oriented electrophoresis', which allows the effective angle of the electric field, and hence the molecular orientation of DNA, to be varied without electrode rearrangement, are also studied. In this case the DNA adopts a 'staircase' configuration showing that the net orientation in a direction is given by the vector sum of the pulses used.

Individual DNA molecules undergoing conventional gel electrophoresis were visualized using a microscope-mounted miniature electrophoresis apparatus. As shown in Fig. 1a-f, molecules initially in a random conformation elongated to form 'hook' structures which aligned with the applied electric field and 'wrapped around' obstacles in the gel. Tension between the two arms of a hook was resolved when one arm became dominant (Fig. 1d, e; molecule 1), pulling the other arm past the obstacle and facilitating coil relaxation to a more random conformation. Recurring cycles of elongation, resolution and relaxation were observed for the duration of the applied steady field. Collectively, these orientational events confirm previously published models<sup>3-6</sup>. The application of a second electric field, oriented at 90° to the first, resulted in a realignment of DNA molecules (Fig. 1g-i). Immediately after field switching, segments of all

molecules began to move in the direction of the new field, and after 39 s, most of the molecules assumed hook structures that were somewhat broader than those found in the steady field case.

Separation by pulse-field electrophoresis depends on the angle between successively applied fields (field angle)<sup>7-11</sup>, with obtuse angles providing superior separation of large DNA molecules. A new electrophoretic effect and its associated method, pulse oriented electrophoresis (POE), was thus used to study the relationship between field angle and DNA conformational changes.

POE has been used to resolve the chromosomes of both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* in a manner analogous to the pulse electrophoretic method<sup>12</sup>. Briefly, POE allows molecular orientation to be varied, without the requirement of electrode rearrangement, by using a series of alternating perpendicular pulses shorter than the orientation time for a given molecule. These pulses are applied such that their predicted effect is to fold a DNA molecule into a staircase-like configuration<sup>9</sup>. The net alignment of each rapidly pulsed molecule is then the vector sum of the DNA segmental orientations corresponding to the 'steps' of the staircase. This alignment can be altered by varying the pulse duration ratio of the two perpendicular fields. Thus, POE emulation of pulse-field electrophoresis using a 90° field angle (Fig. 2a-f) caused molecules to adopt the predicted 'staircase' conformation and exhibit coil dynamics similar to those shown in Fig. 1.

The emulation of an obtuse field angle (~120°) by POE (Fig. 3) resulted in molecules elongating to form broad hooks which also exhibited the staircase effect. These structures collapsed within 6 s of switching fields, whereas roughly 20 s were required for collapse after a 90° field switch (Fig. 2); the apparent coil relaxation time after electric field cessation, however, was about 60 seconds (Fig. 4).

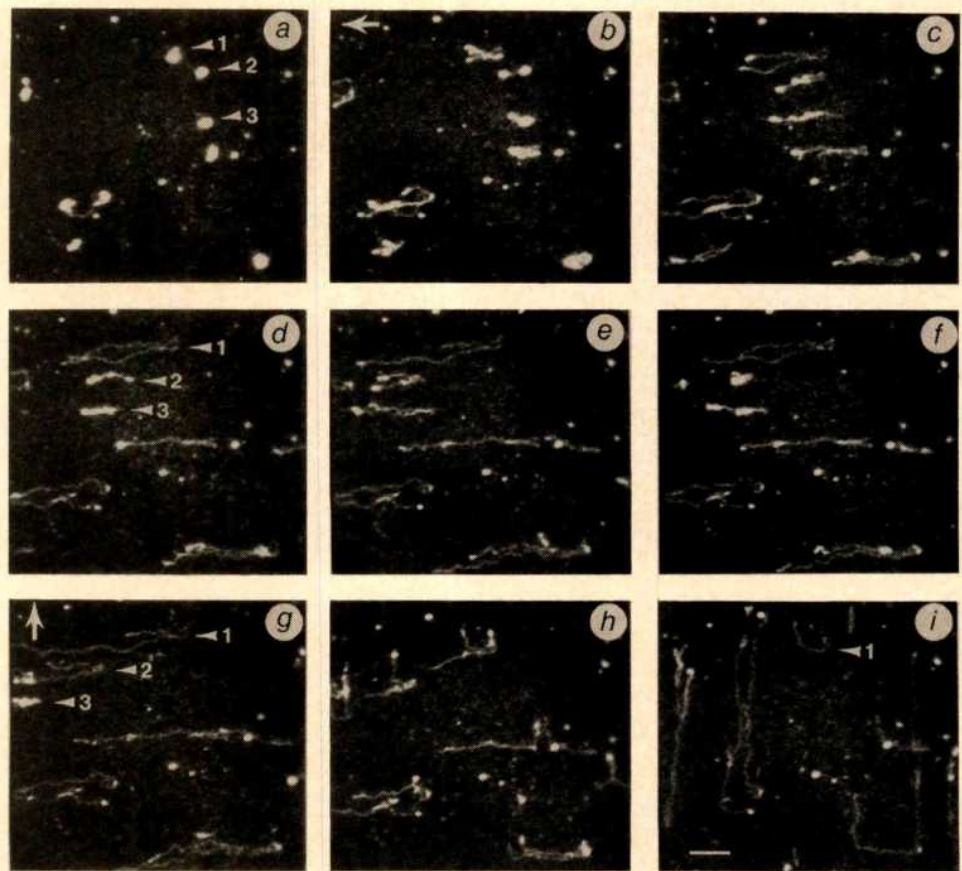
Our results suggest a model for the field-angle-dependent reorientation mechanism of POE that might also apply to pulse-field electrophoresis. Consider the electric field force components generated along a stretched DNA coil axis (constrained by the gel matrix) immediately after the field direction has shifted. If the field angle is 90°, then, on average, both ends of an extended coil will be equally favoured to move in the direction of the new electric field and this inhibits coil relaxation. If the field angle is obtuse, however, then the new electric field will have a component parallel to the elongated coil axis which can



FIG. 1 G bacteriophage DNA molecules, 700 kilobases (kb) long, stained with 4,6-diamidino-2-phenylindole (DAPI) visualized during gel electrophoresis. The images were obtained at 0 s (a), 5 s (b), 16 s (c), 28 s (d), 33 s (e), 44 s (f), 54 s (g), 60 s (h) and 93 s (i) after field initiation. a, The apparent radius of relaxed G bacteriophage DNA (see arrowheads) in a 1.0% low-melting-temperature gel matrix; this was similar to the solution value of  $2.2 \times 10^{-4}$  cm. b, The effect of a uniform electric field ( $3.5 \text{ V cm}^{-1}$ ); the coils move and elongate in the direction of the applied field (arrow). b–f, The continuing movement produces a series of hook structures and thick rod-like coil conformations. g–i, The effect of shifting the field direction by  $90^\circ$  (arrow); the molecules reorient. Bar; 10  $\mu\text{m}$ .

**METHODS.** G bacteriophage was grown as previously described<sup>14</sup>. DNA was prepared by lysing virus in  $1/2 \times \text{TBE}$  buffer (42.5 mM Trizma base, 44.5 mM boric acid, 1.25 mM disodium EDTA) followed by ethanol precipitation; this did not shear the sample. DNA in  $1/2 \times \text{TBE}$  was diluted to  $0.1\text{--}0.2 \text{ ng ml}^{-1}$  in prefiltered 1.0% low-gelling-temperature agarose (Sea Plaque) in  $1/2 \times \text{TBE}$ ,  $0.3 \mu\text{g ml}^{-1}$  DAPI (Sigma), 1.0% 2-mercaptoethanol and held at  $65^\circ\text{C}$ . Samples were mounted using  $3 \mu\text{l}$  DNA-agarose mixture, transferred to a preheated slide, covered, then placed

into a miniature pulse electrophoresis apparatus consisting of a series of discrete platinum electrodes surrounding the square cover slip, wetted with agarose for contact, and connected to diodes to prevent electrode-generated field distortion effects. Samples were pre-electrophoresed for 2–3 min and allowed to relax at room temperature before observation. Field strength was measured using auxiliary electrodes connected to a multimeter. A



Zeiss Axioplan microscope and a C2400-SIT camera (Hamamatsu Corp.) were used with low level excitation light to minimize photodamage during imaging. For each image, eight video frames were digitized, boxcar-averaged and corrected to remove background fluorescence using an IC-1 image processing system (Inovision Corp.).

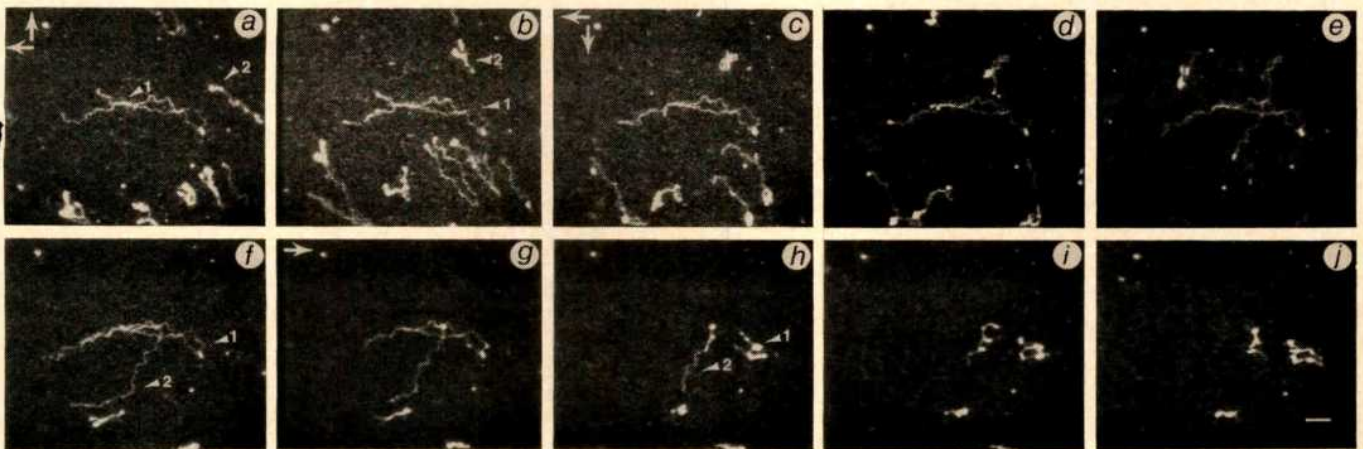


FIG. 2 Bacteriophage G DNA using POE conditions analogous to those for pulse field electrophoresis with a  $90^\circ$  field angle (3, 3–84 s at  $3.5 \text{ V cm}^{-1}$ ; see Methods), followed by field reversal (continuous field;  $3.5 \text{ V cm}^{-1}$ ). a–f, Images obtained at 570 s (a), 600 s (b), 612 s (105 after field switch) (c), 624 s (d), 672 s (e), and 836 s (f) after POE-field initiation. g–j, Images obtained at 2 s (g), 6 s (h), 12 s (i) and 18 s (j) after field reversal. a, The trapped molecule (1) joined by other coils moving into the field from the bottom; b, the newly arrived coils display conformations including hooks and condensed coils. c, After the field switch (arrows), hooks resolve into single strands terminated with a dense region. The newly entangled coil (2) undergoes considerable conformational change from a thick rod (a), to a

stubby hook (b), to a round, dense mass (c). The two trapped coils (d–f) show the 'staircase' effect characteristic of POE. g–j, Pulsing ends and a reversing field is applied, which rapidly unhooks, disentangles and collapses both molecules. Scale bar, 10  $\mu\text{m}$ .

**METHODS.** Microscopy and sample preparation methods are described in Fig. 1. POE utilized a series of short, alternating perpendicular pulses. After a period of time, the polarity of one of the fields was switched. 3, 3–84 s describes a cycle, alternating between a 3 s pulse east–west and a 3 s pulse south–north with an 84 s duration, followed by another 84 s cycle alternating between a 3 s pulse east–west and a 3 s pulse north–south and so on.



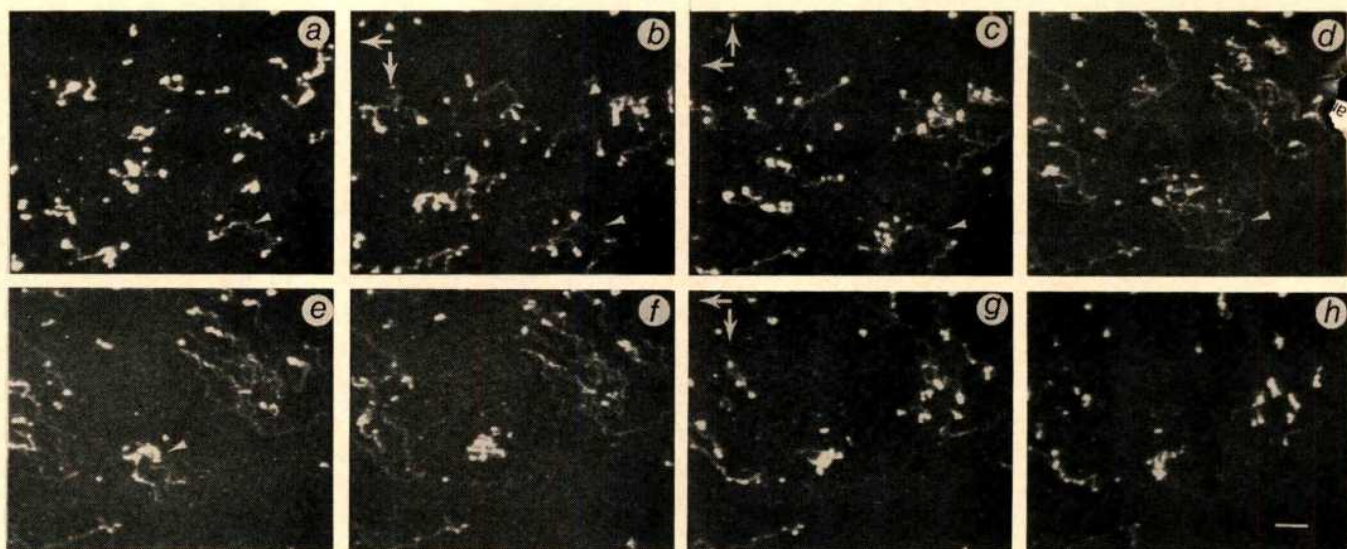


FIG. 3 G bacteriophage DNA electrophoresis with POE conditions emulating an obtuse field angle; 6, 10–80 s, at  $3 \text{ V cm}^{-1}$ . *a–h*, Images obtained 0 s (*a*), 18 s (*b*), 85 s (3 s after field switch) (*c*), 120 s (*d*), 138 s (*e*), 158 s (*f*), 165 s (5 s after field switch) (*g*) and 171 s (*h*) after POE-field initiation. Changes in field orientation are shown by arrows (*b, c, g*). Molecules begin from a

relaxed conformation (*a*) and elongate to a 'staircase' conformation characteristic of POE as the pulsing proceeds (*b–h*). Inversion of the hooks also occurs (*c–e*; arrowhead). Scale bar,  $10 \mu\text{m}$ . Sample preparation and experimental conditions were as described in Figs 1 and 2.

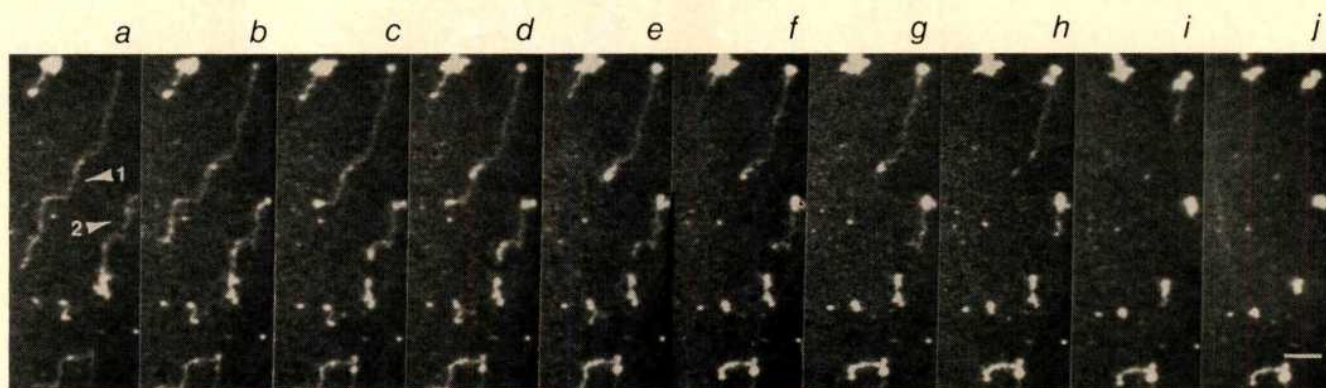


FIG. 4 Relaxation of G bacteriophage DNA molecules in the absence of an electric field. Molecules were initially electrophoresed for 600 s (POE conditions; 3, 5–80 s,  $3 \text{ V cm}^{-1}$ ). Images, obtained at 12 s intervals, show the relaxation of several molecules over a 111-s time span. *a*, 3 s after turning off the applied field. The coils (arrowheads) relax through the same 'staircase'

path that was induced by the applied electrical pulses (as determined by the limits of microscopic resolution). *c*, Note that either one molecule (molecule 2) has fragmented or two molecules have separated. *j*, All coils have relaxed to a round, unelongated conformation. Scale bar,  $10 \mu\text{m}$ . Sample preparation and experimental conditions were as described in Figs 1 and 2.

augment coil relaxation in that direction (in hooks, this is towards the apex). Additionally, the coil ends may not be equivalent, which may also bias motion in both perpendicular and obtuse field situations.

Despite the low sample concentrations used in the electrophoresis experiments, DNA-DNA interactions were also observed. Figure 2, for example, shows a temporarily immobilized DNA molecule (molecule 1) trapping another DNA molecule. Reversal of the electric field ( $180^\circ$  field shift) released both molecules and caused the rapid collapse and eventual

reformation of the hooks. It is probable that similar events occur in reverse-field electrophoresis and this is, indeed, the conclusion of several computer simulations<sup>3,4,6</sup> and spectroscopic studies<sup>13</sup>.

These results show that static-field, pulse-field and pulse-oriented electrophoresis give rise to similar cycles of DNA conformational changes that include hook formation, resolution, collapse and re-formation. Furthermore, it seems that the field angle has a pronounced effect on DNA coil dynamics with perpendicular field angles preventing the rapid collapse of structure after field switching. □

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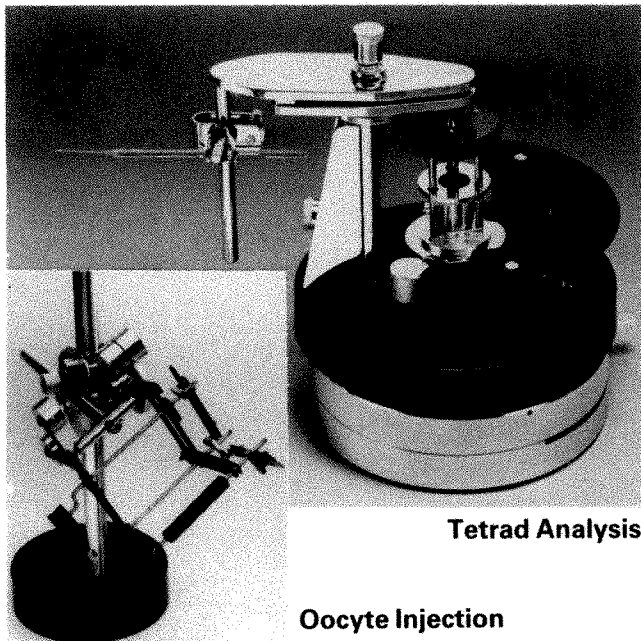
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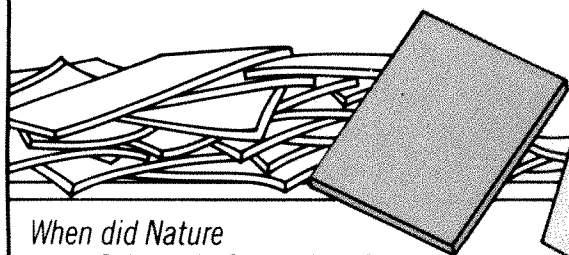
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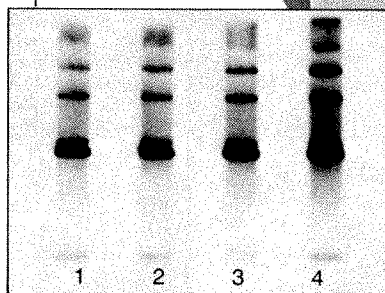
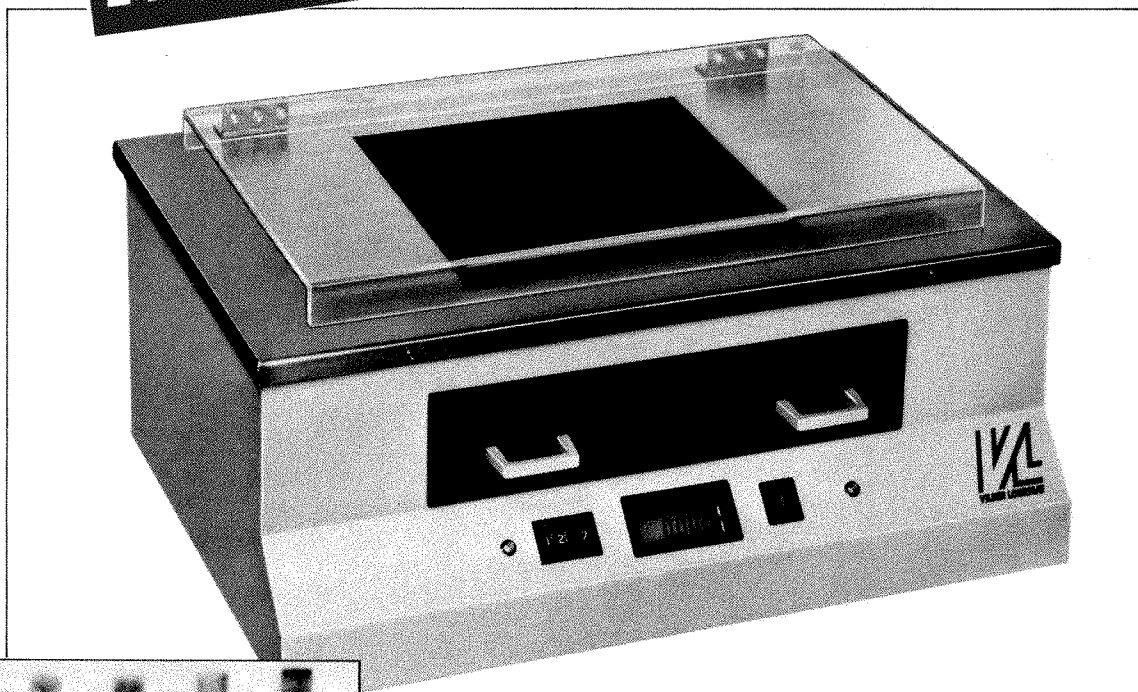
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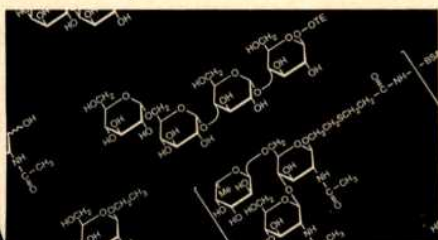
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# Springtime launches for the lab

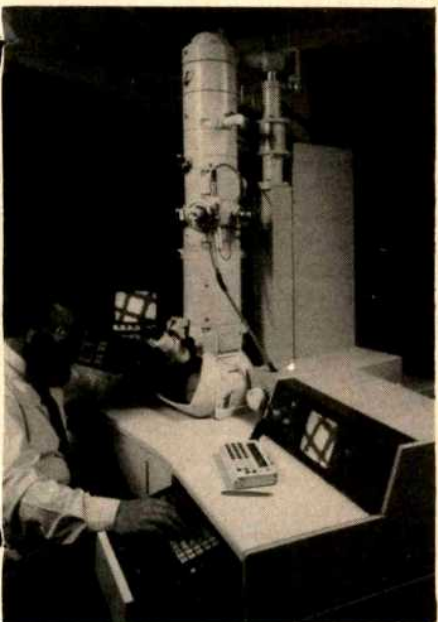
The spring of the year brings a flurry of product introductions: this season's best includes a phenol-free DNA extraction kit, an LC/MS that can handle haemoglobin, and a benchtop robot that does the drudge jobs without complaint.

SIGMA Chemical Company recently announced a special line of highly purified synthetic oligosaccharides and derivative



Bioactive carbohydrates from Sigma Chemical. fragments of biologically active glycoconjugates (*Reader Service No. 100*). The complex **bioactive carbohydrates** have applications in diverse areas of medical and biological research, including substrate specificity studies of glycosidases and glycotransferases, and studies of drug targeting, cell proliferation and oncogenesis. They may also be used as model compounds for the study of complex carbohydrates by NMR spectroscopy, and can be incorporated into liposomes.

Jeol's new model JEM 1200 EXII **electron microscope** was developed specifically to support fundamental biotechnology research and environmental studies involving particle microanalysis (*Reader Service No. 101*). The microscope has a three-stage, six-lens image-forming system, which Jeol says provides high resolution, a wide visual field, low image



Jeol's new transmission electron microscope has a wide magnification range.

distortion and high-contrast image quality at low magnifications. The TEM has a magnification range of 50–1,000,000 ×, and a focus zoom which allows magnification changes without focus changes. Samples may also be measured quantitatively with Jeol's microscope: lengths, angles, and areas of the overall sample can be recorded, and lattice spacing and angles can be determined using diffraction patterns, down to a resolution of 0.14 nm.

Oncor says its new non-organic **DNA extraction kit** produces high-purity, high-yield DNA in four hours, without the use



No fussing with phenol is necessary with Oncor's non-organic DNA extraction kit.

of hazardous organic chemicals (*Reader Service No. 102*). The kit's reagents are qualified on human samples in Oncor's molecular pathology laboratory. The company says the kit provides DNA whose purity compares with phenol extraction: Oncor says the absorbance ratio ( $A_{260}/A_{280}$ ) of the purified DNA falls between 1.75 and 1.90. The kit eliminates the need for dialysis or extraction steps, cutting DNA purification time down from three days to roughly four hours, Oncor says. The proprietary reagents used in the \$150 (US) DNA extraction kit are non-caustic and non-carcinogenic, and can be poured safely down laboratory drains.

## The computing edge

PolyView is the name of a new software system from Varian which aims to make **diode array spectral processing** for liquid chromatography more productive (*Reader Service No. 103*). The PolyView software is designed to work with the Polychrom 9065 diode array detector, as a part of a complete \$23,000 (US) system built around the PC-based LC Star chromato-



PolyView chromatogram analysis software.

graphy workstation. With the Microsoft Windows environment of PolyView, chromatographers can take data from an information-rich detector, and present it in a variety of diverse formats simultaneously on one computer screen. The software has a bidirectional library function: users can search the library for a chromatogram that matches peaks from one obtained experimentally, or search a given chromatogram for similarities to a library standard. Reports are printed automatically at the end of each run. PolyView also calculates Varian's Purity Parameter — the average wavelength weighted by the square of the absorbance — to help chromatographers locate impurities.

Mathor 3, the **technical word processing software** from Novedit, displays scientific formulae and mathematical equations on the computer screen as they will appear in printed form (*Reader Service No. 104*). The software automatically draws and sizes all mathematical symbols, such as square root signs and integrals, to accommodate their contents. Formulas can be inserted on the same line as text, and frequently used mathematical expressions can be stored in Mathor 3's memory to be inserted at the touch of a key. Mathor 3 has five character sets, including the full Greek alphabet, operators and logical symbols, and common physics and mathematics characters. Text may be imported to Mathor 3 from Word and WordPerfect word-processing programs. The FF3,000 (France) Mathor 3 software runs on IBM PC and PS/2 computers equipped with a graphics adaptor card.

The LabSolutions program from The Center for Science Support, Inc. automatically performs the necessary calculations for preparing multi-component solutions and buffers (*Reader Service No. 105*). The \$99 (US) **solution calculation software** uses standard units of weight,



volume, and concentration, and calculates molecular weights from atomic formulas or chemical names. Solution recipes can be stored on disk for retrieval, and printed out for documentation purposes. Specialized routines for mixing and diluting solutions, and for automatically generating linear and geometric dilution series are also included. Users may add their own entries to the built-in databases of chemicals, buffers, acids and bases. The LabSolutions user manual includes chapters on choosing the best buffer, deriving titration equations, and adjusting the pH. LabSolutions is available on 3.5-in. and 5.25-in. diskettes for IBM PCs; a Macintosh version will be released soon.

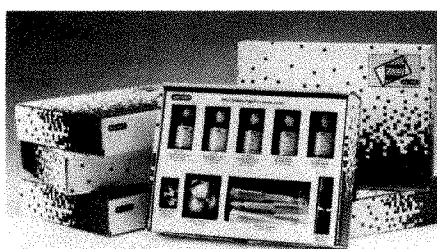
## Popular culture

Laboratory Impex Ltd has a **Mycoplasma detection kit** which it says sniffs out the presence of as little as  $10^3$  organisms contaminating a tissue culture in just three hours (*Reader Service No. 106*). The company's Gen-Probe Mycoplasma TC detection system employs the principle of nucleic acid hybridization: the kit contains a  $^3\text{H}$ -labelled DNA probe homologous to Mycoplasma and Acholeplasma ribosomal RNA. Laboratory Impex says the probe detects all species which commonly infect tissue cultures. Incubating the hybridization reaction for 15–20 hours increases the sensitivity of the test three to five times, according to the company.

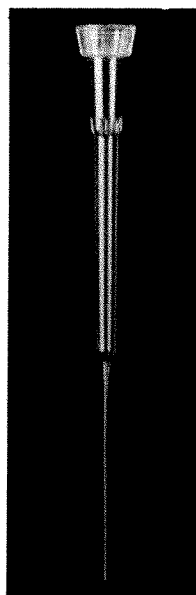
Baxter Healthcare Corporation has a flexible, 25-litre **recovery container** designed to replace carboys and stainless steel tanks in large-scale cell culture (*Reader Service No. 107*). The Lifecell container is composed of several layers of non-toxic, gas-barrier plastic which Baxter says offers stability equivalent to that of glass. The containers require minimal storage space, and can be stacked in tote pans when filled. Baxter says the container is lined with a layer of polyolefin which is non-reactive with biologicals. Two separate tubes with Luer connection ports are attached to the container to facilitate filling and emptying procedures.

## Gel gems

Bio-Rad's roundup of electrophoresis products includes **pipette tips for gel loading** and a **reagent and sample preparation**



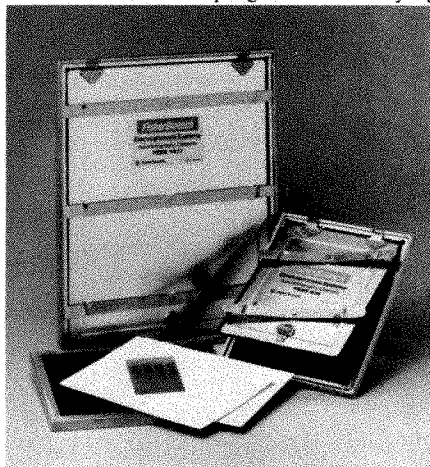
Bio-Rad's kit for readying samples and reagents for electrophoresis.



Bio-Rad's Gel-Loader.

kit (*Reader Service No. 108*). Bio-Rad's Gel Loader disposable capillary pipette tips have a non-wetting surface which delivers samples of between 0.5 and 10  $\mu\text{l}$ . The capillary of each tip is less than 0.35 mm o.d., making them useful for loading 0.25-mm sequencing gels. The 15-mm capillary reaches to the bottom of each well in a gel, eliminating cross-contamination between lanes. Before wielding the pipette, Bio-Rad recommends cleaning up both samples and reagents with its kit which contains all of the materials needed to remove Triton X-100 detergent and SDS from samples, to concentrate 0.5–1.5-ml samples, and to deionize impure urea, formamide, glyoxal or acrylamide. Bio-Rad says use of the kit can prevent band broadening and reduce background levels.

The FisherBiotech division of Fisher Scientific has a new line of **autoradiography cassettes** for developing and intensifying

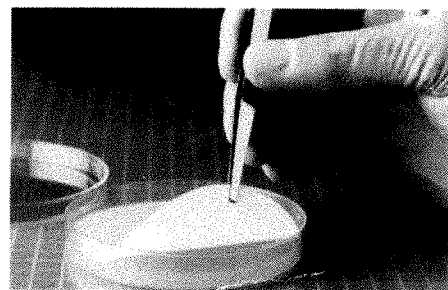


Protective cassettes for autorads from Fisher.

autoradiographs from electrophoretic gels (*Reader Service No. 109*). The cassettes come in sizes of 8 × 10 inches, 14 × 17 inches and 14 × 36 inches. All have stainless-steel frames and felt liners to prevent light leakage and ensure optimal contact between film and screen. The doors of the cassettes have integral locking bars, and Fisher says the cassettes are virtually warp-proof. The 8 × 10-inch cassettes cost \$88 (US); \$96 (US) with screens.

## Probing analyses

Micron Separations, Inc. is giving away an application kit for reprobing its NitroPlus 2000 supported nitrocellulose membranes



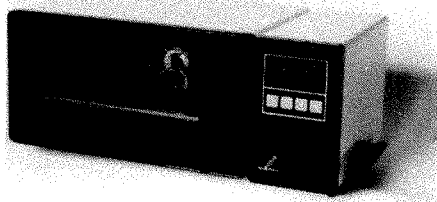
Get a free sample of Micron Separations' supported membranes.

(*Reader Service No. 110*). The **free membrane reprobing kit** contains a sample of MSI's NitroPlus 2000 membrane, two newly developed techniques for reprobing the membrane, and detailed procedures for stripping and reprobing Northern and Southern blots up to six times. Micron Separations says 80–90 per cent of the signal from the target DNA or RNA is retained using the reprobing protocols, with 90–100 per cent of the probe being removed after each reprobing step.

BRL has a new system for simplifying and standardizing the procedure of **labelling DNA probes** with biotin by nick translation (*Reader Service No. 111*). BRL's BioNick labelling system generates small probes which range in size from roughly 50 to 500 base pairs. Because they are small enough to penetrate tissue easily, BRL says the probes are ideal for *in situ* hybridization. The BioNick system employs a new biotinylated nucleotide, biotin-14-dATP, which has a long, 14-atom linker arm that BRL says leaves enough room for streptavidin to bind for the identification of probe-target hybrids. The BioNick kit includes sufficient reagents for labelling 50  $\mu\text{g}$  of DNA: a 10 × dNTP mixture that contains biotin-14-dATP in optimal concentration, a 10 × enzyme solution, stop buffer, autoclaved water, a positive control DNA to monitor performance, and detailed instructions.

## Handy helpers

BioTherm Corporation has a \$2,990 (US) **thermal cycler** for performing enzymatic gene amplification and other variable-temperature protocols (*Reader Service No. 112*). BioTherm's BioOven has an open design which allows it to be used with microtitre plates, microcentrifuge tubes, and a variety of other sample containers.

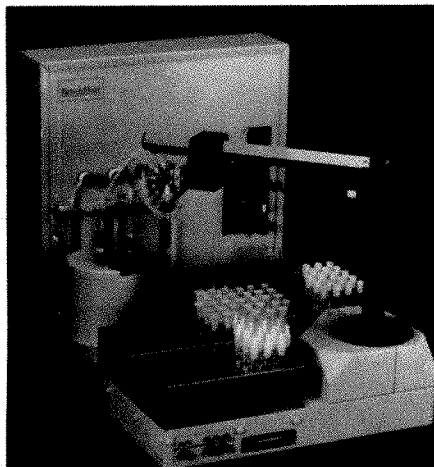


Gene amplification is just one of many applications for the air-heated oven from BioTherm.



The BioOven uses air heat, eliminating water spills and the need for oil layering. BioTherm says the oven heats from ambient temperature to 100 °C in roughly one minute, with a temperature uniformity of 1 °C. The oven's built-in microprocessor allows six independent heating and holding cycles within a given routine; up to eight separate programs can be stored and recalled for later use.

The BenchMate **benchtop robot** from Zymark was designed to free researchers from performing the routine sample preparation tasks required in most laboratories (*Reader Service No. 113*). The BenchMate can be configured to dispense liquids, perform membrane filtrations and solid-phase extractions, and inject samples into an HPLC for analysis. Zymark's \$11,000–18,000 (US) robot takes up just 21.5 × 29.5 inches of bench space, and holds up to 200 standard-sized test tubes. Precision syringes with Zymark's 12-port valving technology dispense up to 10 ml of liquid; with the gravimetric confirmation option, an audit trail can be created to confirm all liquid deliveries by weight. The BenchMate is



Zymark's answer to monotonous lab procedures: BenchMate.

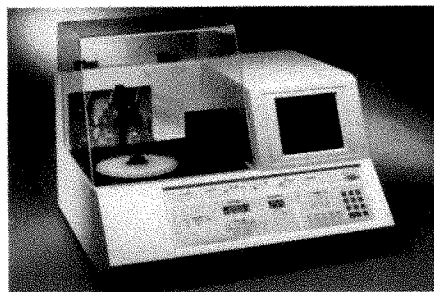
controlled by a 3.5-in diskette which contains a menu-driven operating system for building method files, and can be programmed by direct connection to an IBM PC.

### Analytical technology

Last month Finnigan MAT introduced a **benchtop GC/MS** system which combines complete spectrum separation with detection at the picogram level—the Ion Trap System 40 (*Reader Service No. 114*). The ITS 40 is targeted for the environmental and forensic/clinical markets, where low-level trace identification is important. The system consists of the Varian model 3400 gas chromatograph mounted on a single base plate with the Finnigan ion trap quadrupole mass spectrometer, and controlled by an integrated Compaq Deskpro computer running Finnigan MAT's proprietary software. Finnigan MAT says the

\$85,000 (US) ITS 40 system supports a full-spectrum acquisition rate of up to 10 spectra per second and a scan rate greater than 5,600  $\mu\text{s}^{-1}$ .

Dionex has the latest entrant onto the market of the hottest analytical technique of the year: capillary electrophoresis (*Reader Service No. 115*). The Dionex CES I **capillary electrophoresis** system has detectors for both UV/visible absorbance and fluorescence which are linked to the instrument by fibre optics, bringing the



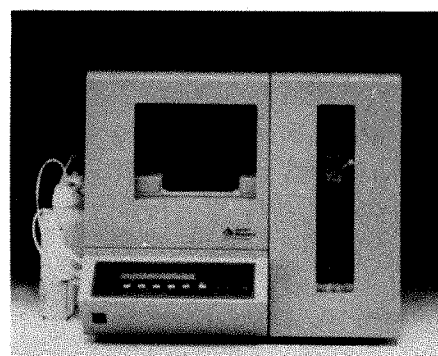
The Dionex system for capillary electrophoresis.

detector closer to the column. The CES I's 40-place autosampler is capable of performing the three most popular injection methods: electromigration, gravity and pneumatic injection. An automatic rinse and refill buffer system allows buffers to be changed during runs, and ensures reproducible electrophoresis by using fresh buffers for each run, says Dionex. The spring-loaded design of the cell means that capillaries can be changed quickly. The \$37,500 (US) system is controlled by menu-driven software and a video display. Results are generated in a chromatography-like format, so the system can interface with any recorder or integrator.

Sciex has developed an LC/MS system especially for the needs of the biopharmaceutical industry (*Reader Service No. 116*). Sciex's API III joins HPLC together with tandem mass spectrometry, through an atmospheric pressure ionization interface that Sciex says outperforms fast-atom bombardment and thermospray ionization methods to allow femtomolar detection. The IonSpray interface desorbs ionic compounds in the solution eluting from the HPLC into the ionization source using field-assisted ion evaporation. The molecular ions are then transferred to the mass analyser through a nitrogen curtain gas. The \$395,000 (US) system can be used to glean sequence information from peptides, proteins and oligonucleotides with molecular weights of over 100,000. Sciex says the API III has been used successfully to analyse haemoglobin.

### Setting it straight

Contrary to an earlier description (see *Nature* 338, 94; 1989), the model 230A HPEC system from Applied Biosystems is not a capillary electrophoresis instrument.



Automated electrophoresis, not CZE.

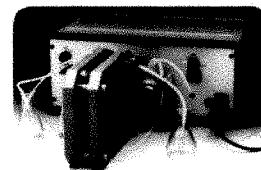
HPEC stands for high-performance electrophoresis chromatograph: the model 230A HPEC is an automated micro-preparative gel electrophoresis system which isolates and quantifies proteins and DNA on a tube gel column using standard electrophoresis protocols (*Reader Service No. 117*). After detection, eluted compounds pass into an integral fraction collector. Applied Biosystems's newly launched analytical capillary electrophoresis system is model number 270A. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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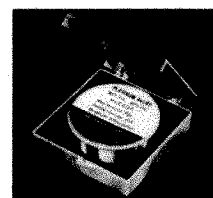
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Reader Service No.46

# The impact of Europe's 1992

Richard Pearson

Business links between Europe and the United Kingdom are increasing and barriers to mobility of labour are coming down. There are new opportunities for both employers and individuals.

As the date for closer European integration draws near, increasing attention is being paid to the possible effects it may have on UK and other European labour markets. International companies are developing management teams which can work across international boundaries. Others are moving into overseas markets for the first time and having to build up a knowledge of local employment conditions and styles of operating. Yet other companies are expanding their recruitment market across national borders.

Individuals are also increasingly moving across borders as horizons of opportunity are broadened from the traditional North American and Commonwealth job markets (*Nature* 319, 84; 1986). For example, we have recently seen a significant inflow of veterinarians into the United Kingdom from Italy and other parts of Europe impacting on the job opportunities of UK graduates. Even those organizations operating solely within the United Kingdom, for example the universities, polytechnics and research institutes, are being affected as European recruiters target the UK labour market. Already Siemens,

a near-universal phenomenon, hitting for example France, Italy, the Netherlands and West Germany alike; in the latter case the downturn is as high as 45 per cent over a decade (see figure), with only Ireland having an expanding youth labour force. We see also a convergence across labour markets with common growth occupations at high skill levels and in the services sector, and declining opportunities for the unskilled and manual workers.

Because of differences in structure and content, comparisons of the numbers graduating have to be made with care. For example, while fewer students in the United Kingdom are enrolled in higher education pro rata to population size, the numbers graduating compare more favourably because of the lower drop-out rates, and although the United Kingdom does not compare so well in terms of engineering it does rather better in the sciences. In terms of the balance between supply and demand we see a segmented market in many countries with high levels of graduate unemployment in the Netherlands, Spain, West Germany, Italy and Ireland coexisting with shortages of engineers, information technology specialists, and business studies graduates.

So how easy will it be to recruit in Europe? The free mobility of labour in the Community means work permits are no longer a problem, although Portugal and Spain will not be included until 1993. Another change about to make itself felt is the mutual recognition of qualifications. Mutual recognition for pharmacists has recently been achieved, but only after 16 years of negotiations, and that for architects after 17 years. A more specific problem will be knowing where to go to recruit and being able to make judgements about the European graduates. For example, while in the United Kingdom the careers services actively help recruiters by 'selling' their graduates, in many parts of Europe such services are rare and in some cases direct recruitment is banned on campuses. In these cases use has to be made of more general national employment services. Recruitment is more usually carried out through personal links with academics, student work placements and direct advertising in the national newspapers and professional journals. Speculative applications from students are also much more common. In Italy, family and personal contacts are also important, factors reinforced by the much greater tendency of students to live at home and go to local

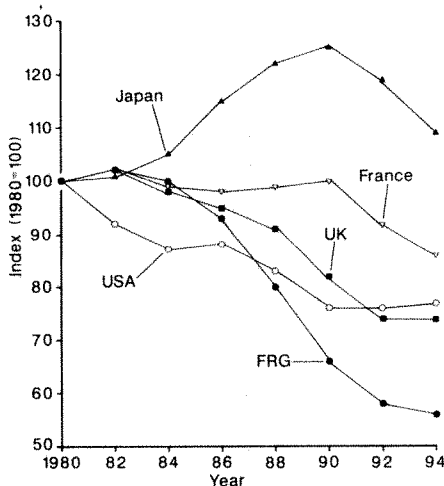
universities, a practice which is common in continental Europe. Living away from home seems to be peculiar to Britain and to students attending national, prestigious institutions in Europe. A further complication for the outside recruiter is the problem of judging the relative merits and qualities of institutions, for example, comparing Louvain with Berlin and Barcelona.

New employment strategies will also be required for the European graduate, who will typically be 4-5 years older than the UK equivalent and will have come from a broader-based, although still specialist, degree course. Different selection tests may be needed, certainly less reliance can be placed on extracurricular activities, which are far less common, and on academic references, which are not normally available on the continent. As most European graduates will be aged 26-28, because of their longer degree courses and national service, they are likely to be more mature and capable than the 21-year-old UK graduate. Initial jobs are therefore likely to be at a higher level in an organization. Induction and initial training needs are therefore also likely to be different, as will the starting salary. In part these differences help explain the discrepancies that are quoted between UK and European graduate starting salaries.

Many challenges face the British recruiter looking to Europe as a new recruitment source. At the same time European recruiters are starting to target the United Kingdom so we will not have exclusive use of the home market. Competition is likely to be greatest in the case of the extended engineering degrees, some of the applied sciences and business courses, particularly where students have a foreign language. Few European recruiters are interested in the generalist graduate. A dilemma for our careers services will be whether, in the interests of serving their prime customers, the students, they encourage and welcome European recruiters on to the campuses.

This increasing internationalization of business and labour markets will increase mobility both into and out of the United Kingdom. European recruitment is not, however, going to be an easy solution to UK shortages. The challenge for the 1990s will be to ensure that European integration offers new opportunities for individuals and employers and does not just mean a new brain drain.

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton, BN1 9RF, UK.



Index of populations of 16-18-year-olds, taking 1980 as the baseline (index 100). Source: *International Science and Technology Update* (NSF, Washington, 1986).

Thompson and Philips are among the regular recruiters in the United Kingdom.

In the United Kingdom skill shortages are going to get worse, because the downturn in the number of young people entering the labour market over the next five years will be even more disruptive if the planned economic growth continues. So are things easier in Europe? The first point is that the demographic downturn is

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## INSTITUTE OF VIROLOGY AND ENVIRONMENTAL MICROBIOLOGY (OXFORD)

### RESEARCH VACANCIES

## MICROBIAL GENETICS, MOLECULAR BIOLOGY AND MICROBIAL PLANT ECOLOGY

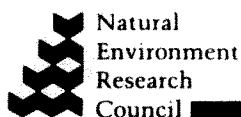
With the expansion of the remit of the NERC Institute of Virology to include environmental microbiology, and the change of the name to the INSTITUTE OF VIROLOGY AND ENVIRONMENTAL MICROBIOLOGY, a new program of research in environmental microbiology has been launched.

Three new staff appointments have been created, two at the post-doctoral, (HSO), level and one at the post-graduate, (SO), level. We are looking for highly motivated individuals, with experience in prokaryotic genetics and molecular biology who would be interested in developing a research career in this important area. Knowledge of microbial ecology would be an asset. The initial research objectives of the group include the quantification and study of the fate, transfer and persistence of genetically engineered bacteria and their DNA in the environment following release onto plant surfaces, thereby leading to an understanding of the potential risk and environmental impact.

Salary will be in the range of £8,574-£10,994 for Scientific Officer appointments and £10,026-£13,460 for Higher Scientific Officer appointments. Starting salary depends on experience and qualifications. Staff of the Council are not Civil Servants but their pay and conditions are similar to those on the Civil Service. There is a non-contributory pension scheme.

Application forms are available from the **Administration Officer, Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, OX1 3SR. Telephone: Oxford (0865) 512361.** Informal enquiries may be made to Dr Mark J Bailey, at the above address, for further details of the posts available.

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(8902)A

### FACULTY POSITION IN MARINE REMOTE SENSING

The Applied Ocean Science Program at the University of Delaware's College of Marine Studies invites applications for tenure track faculty position to start anytime after September 1, 1989.

Requires Ph.D in the physical sciences or engineering, with strong background in theoretical and experimental work on hydrologic optics and/or ocean/coastal remote sensing desirable. Involves teaching at the graduate level, and development of an independent, funded research program on the optical and physical properties of particles and dissolved substances in estuaries and electromagnetic wave interaction with surface films, waves and particles. Opportunity to interact with ongoing research in remote sensing of wetlands and estuarine productivity; coastal circulation and fronts; air-sea interaction; spectral/spatial analysis of satellite imagery; electromagnetic and sound wave interaction with suspended particles; and Laser Doppler characterization of marine plankton.

Send resume with names and addresses of three references to: **Dr. Stephen C. Dexter, College of Marine Studies, University of Delaware, Lewes, DE 19958.** Closing date: July 1, 1989. *The University of Delaware is an Affirmative Action/Equal Opportunity Employer.* (NW3558)A

### MEDICAL RESEARCH COUNCIL LABORATORY OF MOLECULAR BIOLOGY, CAMBRIDGE

## Research in Organic Chemistry

Applications are invited for **post-doctoral** and **graduate positions** on the staff of the laboratory to join a research group working in the peptide and oligonucleotide field.

A PhD, first degree, or equivalent qualification in chemistry or a related subject is required. For both positions, experience in peptide synthesis or general organic synthesis and an interest in computer-controlled instrumentation would be an advantage. The post-doctoral position (ref D/5819) is initially of three years duration, subject to confirmation after the first year; salary within the range £11,070-£14,500 per annum (under review). The graduate appointment (ref D/2272) will be made on the Senior Technician scale which rises to a maximum of £12,347 per annum, with tenure after a six months probationary period. MRC Pension Scheme option, staff restaurant, and sports facilities.

Both positions are available immediately. Applications, including a curriculum vitae and the names of two professional referees should be sent quoting the appropriate reference by 30 April 1989 to:—

**The Personnel Officer  
MRC Centre  
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Cambridge  
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## DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

### Postdoctoral positions

#### Postdoctoral Research assistant 1a BR/287

#### CRC Growth Factors Research Group Growth and differentiation factors

Applications are invited for a postdoctoral 1a position in the CRC Growth Factors Research group. The successful applicant will be involved in the molecular cloning and structural characterisation of genes encoding novel polypeptide growth and differentiation factors active in early mammalian development. Techniques employed include DNA library construction, DNA sequencing, polymerase chain reaction and eukaryotic expression systems. Appropriate experience in these areas would be advantageous. The post is supported until December 1993 in the first instance. Salary scale: £9,865–£15,720 depending upon age and experience.

#### Postdoctoral Research Assistant 1a BR/288

#### Mammalian transgenesis

Applications are invited for a postdoctoral 1a position, supported by the AFRC Transgenic Animals Programme, for the exploitation of yeast gene control systems in transgenic animals. A background in yeast or mammalian gene expression and interest in acquiring experience in transgenesis techniques would be advantageous. The post is supported for three years from the date of appointment. Salary scale: £9,865–£15,720 depending upon age and experience.

Informal inquiries regarding both positions may be directed to Dr. John K Heath (tel. 0865/275721)

#### Postdoctoral Research Assistant 1a BR/289

#### Yeast/mammalian molecular genetics

Applications are invited for a postdoctoral 1a position, supported by the Wellcome Trust, for the molecular analysis of genes encoding sequence specific DNA binding proteins involved in centromere function in yeast, and their mammalian homologues. A background in yeast or mammalian molecular genetics would be an advantage. The post is supported for three years from October 1989. Salary scale: £9,865–£15,720 depending upon age and experience.

Informal inquiries may be directed to Dr. Jane Mellor (tel. 0865/275236)

Applications, quoting job number, a full c.v. and the names and addresses of two referees to: The Administrator

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DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD, OXFORD, OX1 3QU.

## UNIVERSITY OF ST ANDREWS

### Department of Biochemistry and Microbiology POSTDOCTORAL RESEARCH ASSISTANT, GRADUATE RESEARCH ASSISTANT AND Ph.D STUDENTSHIP AIDS VACCINE DEVELOPMENT

Applications are invited for the above posts, funded by the MRC AIDS Directed Programme for three years, to develop and test the ability of novel immunogens to induce immunity to human immunodeficiency virus (HIV). The project will involve the construction of human adenovirus recombinants expressing HIV and SIV proteins, incorporation of the purified proteins into Solid Matrix-Antibody-Antigen Complexes and assessment of their ability to evoke humoral and cell-mediated immune responses. Successful candidates will join an active, well funded research group with excellent facilities for molecular virology.

For the postdoctoral and graduate research assistant positions experience in immunology (particularly T cell responses), virology or molecular biology would be particularly advantageous. Appointments can commence on 1st June 1989 or at a mutually agreed later date and will be on Research Assistant 1A scale £9,865 to £11,680 per annum, or 1B scale £8,675 to £10,460 per annum. The Ph.D studentship is to commence on 1st October 1989 and priority will be given to high quality candidates rather than background degree.

Further information can be obtained by telephoning Dr R E Randall or Dr R T Hay (0334 76161). Applications (two copies preferably in typescript) with the names of two referees should be sent to the **Director of Personnel Services, The University, College Gate, St Andrews, Fife KY16 9AJ** to arrive by 30th April 1989. (8923)A

## University of Minnesota Department of Pharmacology

### POSTDOCTORAL AND RESEARCH ASSOCIATE POSITIONS

The Department of Pharmacology, University of Minnesota Medical School, has Postdoctoral and Research Associate positions available in several fields. Our faculty members are well-funded and direct strong interdisciplinary research programs.

Positions are open to applicants upon receipt of a Ph.D. degree.

Areas of emphasis include:

**Molecular biology**, biochemistry, immunology, and neurobiology-neurochemistry

**Molecular cloning**, opioid receptor, receptor, and chemical biochemistry

**Intracellular electrophysiology**, ion channels

**Drug abuse**, neuropharmacology

**Biochemical/molecular pharmacology**

**Cancer chemotherapy**, immunopharmacology, drug metabolism, enzymology, and anti-viral drugs

Interested applicants should send a curriculum vitae, statement of research interests, and the names of three references to:

**Dr. Horace H. Loh, Professor and Head,  
Department of Pharmacology,  
3-249 Millard Hall,  
435 Delaware Street SE,  
Minneapolis, MN 55455.**

*The University of Minnesota is an equal opportunity educator and employer, and specifically invites and encourages applications from women and minorities.* (NW3555)A

## MEDICAL RESEARCH COUNCIL

### POST-DOCTORAL MOLECULAR GENETICIST

Applications are invited from suitably qualified applicants for a non-clinical scientist to join the Development Genetics Group on an established project studying the molecular basis of mutations affecting melanocyte development in the mouse. The work will involve DNA cloning, sequencing and manipulation, cell biology and mouse genetics, and experience in one or all of these areas will be an advantage. The position is available immediately for up to 3 years on a scale of £9260–£14500 (increase pending).

Enquiries should be made to Dr Ian Jackson, and applications containing the names and addresses of two referees should be sent to **The Unit Administrator, MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU**, Telephone No 031-332 2471.

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(8909)A

## University of Cambridge

### Department of Biochemistry

### PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY

Applications are invited for a postdoctoral research assistantship for work on the biochemistry and molecular biology of Photosystem I polypeptides involved in the ferredoxin-plastoquinone reductase system of cyclic phosphorylation in chloroplasts. The post is funded by AFRC and is available now for a period of up to 3 years, with a salary in the range £9,865–£12,760 according to age and experience.

Applications (with curriculum vitae and names of two referees) should be sent to **Dr. D.S. Bendall, Department of Biochemistry, Tennis Court Road, Cambridge, CB2 1QW**, as soon as possible. Further details from Dr. Bendall, Tel: 0223-333631/333630. (8899)A

# Infection Research

## Two Senior Appointments c £24,000 + car + benefits : Cheshire

As part of a major commitment to research in infection ICI wishes to appoint TWO SENIOR RESEARCH SCIENTISTS to work on exciting and challenging projects in multidisciplinary Project Teams within our Infection Research Programme. One position is for an experienced microbial biochemist to initiate research exploring new therapeutic targets for infection control in bacterial pathogens. The other is for an equally experienced scientist to investigate host defences and/or pathogenesis in an exploratory project aimed at the discovery of new approaches to the therapy of infections.

Candidates will have several years postdoctoral research experience in relevant aspects of microbial biochemistry or host immunity and the biology of infection processes. A knowledge of microbial genetics, in addition to microbial physiological

processes, will be an advantage for applicants for the biochemical post. An interest in chemotherapy of infection is essential.

Applicants should have had significant experience of supervising other research workers – juniors, graduates, PhDs – and must be able to provide evidence of working successfully in a team.

At ICI you will have the opportunity to fulfil your potential, utilise your scientific talent and progress your career in a rapidly expanding international business. An excellent benefits package includes pension, profit share, private healthcare and relocation assistance to this attractive part of the country.

**Please write with full c.v. and salary details, quoting reference AMS to: The Personnel Department, ICI Pharmaceuticals, Mereside, Alderley Park, Nr Macclesfield, Cheshire SK10 4TG.**



**Pharmaceuticals**

(8918)A

## Karolinska Institute Department of Molecular Biology POSTDOCTORAL POSITION

### Molecular Biology of Membrane Protein Assembly

We have an immediate opening for a postdoc interested in working on problems related to protein sorting and membrane protein assembly (see PNAS **85**, 3363-3366 and BBA **947**, 307-333). Our department is wholly focussed on this field, using current methods of molecular and cell biology such as site-specific mutagenesis and *in vitro* transcription-translation assays. Applicants should therefore have a good background in molecular-biology or microbiology.

Salary is 5500 Skr per month tax free (approx £500); in addition, apartment rent, health insurance, and travel costs are covered. The Department of Molecular Biology is a part of the Karolinska Institute and is located alongside the Karolinska Center for Biotechnology in Huddinge, south of Stockholm.

Please address all enquiries to: **Dr. Gunnar von Heijne, Department of Molecular Biology, Karolinska Institutet, Huddinge Hospital-K87, S-141 86 Huddinge, Sweden. Phone: Int +46-8-774 7699 (W6028)A**

## UNIVERSITY OF COLORADO, SCHOOL OF MEDICINE, DENVER, COLORADO DEPARTMENT OF PHARMACOLOGY CHAIRPERSON

Applications and nominations are invited from candidates for the Chairperson of the Department of Pharmacology. Individuals with a strong commitment to medical and graduate education and distinguished accomplishments in basic research are encouraged to apply.

Please send curriculum vitae and names of references to:

Dr. Frederick C. Battaglia  
Chairperson, Pharmacology Search Committee  
Department of Pediatrics, School of Medicine  
University of Colorado Health Sciences Center  
4200 E. 9th Avenue, Denver, CO 80262

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Biology

## Drug Delivery

Smith Kline and French Laboratories, a leader in pharmaceutical research, is currently building a strong multidisciplinary drug delivery department of approximately 30 scientists. This group, located in suburban Philadelphia, will be investigating membrane and transport processes that can be utilized for the delivery of conventional and biotech drugs, and the design of therapeutic systems to meet the biological needs of specific drugs and diseases.

We are therefore seeking outstanding individuals to work in the following areas:

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- Drug Targeting
- Drug Carriers

Ph.D. and non-Ph.D. scientists with backgrounds in either cell biology, physiology, biochemistry, biophysics, or chemistry, and a desire to be part of a highly interactive team, are invited to apply. Previous experience of drug delivery research and development is not a prerequisite.

Based in a new, state-of-the-art research facility near Valley Forge, Smith Kline and French provides a stimulating and challenging environment in which to grow and excel. We offer an attractive compensation/benefits and relocation package. For confidential review forward your C.V. and the names of three references to: Terry Gallagher, T103, Smith Kline & French Laboratories, Research and Development, P.O. Box 1539, King of Prussia, PA 19406-0939. We are an Equal Opportunity Employer, M/F/H/V.

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## **Research Co-ordinator Science Planning Unit**

**Salary circa  
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This large Postgraduate Medical School which includes the Institutes of Cancer Research, Child Health, Dental Surgery, Neurology, Ophthalmology and Psychiatry, and the National Heart and Lung and Hunterian Institutes, is establishing a new science Planning Unit to assist in the development and implementation of an academic plan.

The Research Co-ordinator, a graduate preferably with experience in data handling/computing, will be responsible for the day to day running of the project, maintaining close links with Institute staff and the Regional Deans in Medicine and Dentistry.

Further information and application forms are available from Jane M. Jones, Federation Secretary, BPMF, 33 Millman Street, London WC1N 3EJ (Telephone 01-831 6222) to whom applications should be forwarded by 20 April 1989. (8847)A

## **SYSTEMS NEUROSCIENCE, CELLULAR NEUROSCIENCE, MOLECULAR NEUROSCIENCE; FACULTY POSITIONS DEPARTMENT OF NEUROBIOLOGY AND BEHAVIOR —SUNY STONY BROOK:**

The Department of Neurobiology and Behavior of SUNY Stony Brook is continuing its search for candidates for tenure-track, state-funded positions beginning as early as academic year 1989/90. The Department, which currently consists of 15 members, is undergoing significant redevelopment, and has the resources to grow to 20 members within the next 5 years. Presently available positions are at the Assistant Professor level although an appointment at the rank of associate Professor could be considered. Rank and competitive salaries will be commensurate with qualifications. All applicants should have a minimum of 2 years of postdoctoral experience. Candidates will be expected to develop a vigorous independent research program, and as such ample space and start-up funds will be provided.

Applicants should submit a curriculum vitae, statement of research interests, and names and addresses of three references to: **Dr. Lorne Mendell, Chairman, Department of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY 11794-5230.** SUNY Stony Brook is an affirmative action/equal opportunity educator and employer. AK 60. (NW3516)A

## **AFRC INSTITUTE FOR ANIMAL HEALTH**

Compton, Newbury, Berkshire. RG16 0NN

## **A Head of Division is required for Immunology and Pathology**

Following a major restructuring of the Institute two major divisions: Molecular Biology, and Immunology and Pathology, and two smaller divisions: Environmental Science, and Epidemiology, have been formed. We are seeking to appoint a Head of Division of Immunology and Pathology.

The post will be located at Compton but the successful applicant will be expected to manage an integrated programme across the two major sites at Compton and Pirbright and the Neuropathogenesis Unit in Edinburgh. Opportunities exist to work on a number of endemic and exotic diseases of farm animals and develop fundamental and applied programmes. The Head of Division will plan financial provision, control budgets, encourage collaborative links both within the UK and overseas and take an active role in the management of the Institute.

The successful candidate will have the opportunity to introduce and develop new research topics and appoint to a number of new posts. He/she will be responsible to the Director of Research, Professor F. J. Bourne.

Applicants will have a higher degree and broad experience in modern immunological techniques, distinction in a relevant area of immunological research and an ability to manage scientific personnel.

The appointment will be at Unified Grade 5 with a salary on the scale £28,170-£31,602 with opportunity for further, performance-related increments to £36,786. There is a non-contributory superannuation scheme.

Application form and further details are available from the **Personnel Officer, AFRC Institute for Animal Health, Compton, Berks, RG16 0NN.**

Closing date: 23 April 1989.

(8861)A

## **ANIMAL BIOTECHNOLOGY CAMBRIDGE LTD**

### **SENIOR MOLECULAR BIOLOGIST**

ABC Ltd is a rapidly expanding company in the forefront of the development of advanced transgenesis for the Pharmaceutical, Food and Livestock Industries. We have extensive in-house expertise in animal embryology, *in vitro* culture, stem cell technology and micro-manipulation.

We are looking for an accomplished Molecular Biologist with commercial awareness, to take responsibility for all molecular aspects of our programme and to participate in the development of contracts with our clients.

The successful candidate will have experience in recombinant DNA technology, mammalian gene cloning, and tissue culture. He or she will be responsible for preparing research proposals, setting up projects and managing them. In return we offer a competitive salary and fringe benefits, and the opportunity to work with a well-funded and dedicated team in a stimulating R&D environment.

*Please send your application, including CV and detailed description of previous relevant experience to:*

**Dr Martin Evans**

**Director of Transgenesis**

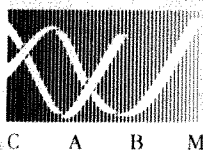
**Animal Biotechnology Cambridge Ltd**

**Animal Research Station**

**307 Huntingdon Road, Cambridge CB3 0JQ**

**Telephone: (0223) 277222 (8860)A**





Postdoctoral positions are now available at the Center for Advanced Biotechnology and Medicine, a newly established molecular biology research program jointly administered by Rutgers University and the University of Medicine and Dentistry of New Jersey

and funded by the N.J. Commission on Science and Technology. Competitive stipends and benefits are provided.

**Structural Biology:** Three positions examining the molecular basis of cancer. NMR Spectroscopist: to develop 2D- and 3D-heteronuclear experiments to characterize the structure and dynamics of proteins in solution. Theoretical Protein Chemist: to implement and develop methods for determining protein structures from NMR data using distance geometry and conformational energy calculations and to aid in molecular modeling and design of transforming growth factors. Experimental Protein Chemist: to perform NMR structural studies, isotope enrichment, and purification of transforming growth factors and mutant analogs. Send CV, summary of research interests, and names of three references to Dr. Gaetano Montelione.

**Molecular Genetics:** Two positions in experimental protein design. Mutagenesis approaches will be used to study problems in molecular recognition, especially ligand:receptor and enzyme:substrate interactions. Applicants should have a strong grounding in chemistry, biochemistry, or biophysics and a desire to apply the techniques of molecular genetics to problems in structural biology. Send CV, a short summary of research interests, and names of three references to Dr. Stephen Anderson.

CABM, 675 Hoes Lane, Piscataway, NJ 08854-5635.

An Affirmative Action-Equal Opportunity Employer.



(NW3552)A

## INSTITUTE OF VIROLOGY AND ENVIRONMENTAL MICROBIOLOGY (OXFORD)

### RESEARCH VACANCIES MOLECULAR BIOLOGY AND ECOLOGY

Applications are invited for research positions in both molecular biology and ecology.

The subject areas are as follows:

#### At post-doctoral, (HSO) level

- 1 Development of novel recombinant retroviral vaccines.
- 2 Release of genetically marked bacteria into the environment, genetic and ecological considerations.

#### At post-graduate, (SO) level

- 1 Environmental investigations into the fate and persistence of phyloplane bacteria.
- 2 Ecology of insect viruses and their use as pest control agents.
- 3 Molecular biology of RNA virus replication.

Applicants should preferably have experience in molecular biology and/or ecology.

Salary will be in the range of £8,574-£10,994 for Scientific Officer appointments and £10,026-£13,460 for Higher Scientific Officer appointments. Starting salary depends on experience and qualifications. Staff of the Council are not Civil Servants but their pay and conditions are similar to those of the Civil Service. There is a non-contributory pension scheme.

Application forms are available from the **Administration Officer, Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, OX1 3SR.** Telephone: Oxford (0865) 512361.

The Natural Environment Research Council is an equal opportunity employer



(8921)A

## AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH EDINBURGH RESEARCH STATION POSTDOCTORAL RESEARCH IN TRANSGENIC ANIMALS

The Research Station is a leading centre in transgenic biology (see Nature, 328, 530-532; 331, 70-72; Bio-technology 6, 179-183). We have embarked on a major expansion in this area and are applying gene transfer techniques in both laboratory and farm animals to critical problems of gene regulation, physiology and development. Within this programme the following postdoctoral research positions are still available.

1. Control of Milk Protein Gene Expression. The research will define the DNA sequences and their interaction with specific transcription factors that underly tissue-specific expression in the mammary gland. The work will be carried out *in vitro* and in transgenic mice. The person appointed will have experience in recombinant DNA techniques. Contact John Clark, 031-667-6901.
2. Manipulation of Milk Composition. The project will involve introducing genes encoding caseins and genetically engineered derivatives into transgenic animals and assessing the effects of their expression on milk composition and properties. The work will be carried out in collaboration with the Hannah Research Institute, Ayr. The person appointed will have experience in recombinant DNA techniques. Contact John Clark, 031-667-6901.
3. Germline transformation of poultry. A molecular biologist is required to join a project to develop a method to produce transgenic poultry by injection of DNA at the single cell stage, followed by *in vitro* embryo culture. The project will involve developing methods to detect the integration of injected sequences and an investigation of the possible use of episomal vectors. For further information contact Helen Sang, 031-440-2726.
4. Increased efficiency of gene transfer in sheep. The objective is to use the polymerase chain reaction to study the integration of DNA sequences in the early embryo after micro-injection. Methods will be developed for 1), selecting transgenic embryos before transfer to recipients and 2), investigating factors that influence the frequency of integration. Experience of working with mammalian embryos would be an advantage. Contact Ian Wilmut, 031-440-2726.

The Research Station occupies new laboratories within and just outside Edinburgh and carries out basic research in animal genetics, molecular biology and physiology; it has close links with biology departments within Edinburgh University and, in addition, will retain facilities within the newly formed interdisciplinary Research Centre.

Condition: All posts are tenable for three years in the first instance. They are all at the HSO grade, except post 3 which may be offered at the SSO grade. Salary in HSO scale £10,026-£13,460 (HSO), 22 days annual leave; £12,445-£17,032 (SSO), 22 days annual leave.

Application forms (specify which posts) can be obtained from **Mrs. L. Hunter, Personnel Officer, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS**, and should be completed by 30th April, 1989. (8926)A

## COMMISSARIAT A L'ENERGIE ATOMIQUE INSERM Département de Biologie U150 EICOSANOÏD RESEARCH

A post-doctoral position is available in a joint project between CEA (Commissariat à l'Energie Atomique) and INSERM (Institut National de la Santé et de la Recherche Médicale) for an individual interested in learning and developing techniques of immunoanalysis for eicosanoids metabolites and their enzymes of biosynthesis (the project will involve enzyme purification, obtention of monoclonal antibodies, preparation of enzymatic tracers). The study will be focused on the development of immunocytochemistry using these reagents. Application of these techniques to blood and vascular cells will also be performed.

The candidate should also have been involved in one of these topics: Analytical Biochemistry, Biochemistry, Pharmacology, Immunochemistry. Send or fax (1) 69 08 73 00 letter of interest, CV and the names of three referees to:

**Dr P. PRADELLES**  
Département de Biologie  
Section de Pharmacologie et  
d'Immunologie  
Bat 136, CEN Saclay  
91191 GIF sur YVETTE Cédex  
FRANCE

**Dr. J. MACLOUF**  
INSERM U150, CNRS URA 184  
Hôpital Lariboisière  
6, rue Guy Patin  
75475 PARIS Cédex 10  
FRANCE

(W6021)A

## The Center for Advanced Research in Biotechnology (CARB)

### MOLECULAR MODELING OF ANTIBODY-ANTIGEN INTERACTIONS

IGEN Inc. wishes to appoint a post-doctoral CARB-IGEN fellow to work on the development of computer algorithms for determining the mode of interaction of antigens with antibody molecules. The successful applicant will be based primarily at the new CARB facility at the Shady Grove Campus of the University of Maryland in Rockville, Maryland, outside Washington, DC. He or she will also interact closely with the IGEN team developing catalytic antibody technology. In addition, collaboration with the antibody engineering group of Dr. A.R. Rees at the University of Oxford will be possible.

The successful candidate will have experience in the development of software for the computer modeling of protein structure and function, and will have a keen interest in meeting the challenges posed by the antibody antigen recognition problem.

Please send a current CV, and a list of 3 individuals who will serve as references to: **Dr. John Moulton, CARB, 9600 Gudelsky Drive, Rockville, MD 20850.** The University of Maryland is an equal opportunity employer.

*CARB is a joint venture between the University of Maryland and the National Institute of Standards and Technology. CARB's goal is to build a center of research excellence in the area of protein structure, function, engineering and design. Molecular modeling is an important component of this program, and is supported by a number of Silicon Graphics workstations and an Alliant minisuper-computer.*



### CATALYTIC ANTIBODIES

IGEN Inc. is seeking a post-doctoral scientist with experience in computational chemistry of small molecules to join its team on the design of transition state analogues for use in the production of catalytic antibodies. Knowledge of protein structure and function would also be an advantage. The successful candidate should be able to participate in the academic activities of CARB.

Please send a current CV, and a list of 3 individuals who will serve as references to: **IGEN Inc., Human Resource Manager, 1530 East Jefferson Street, Rockville, Maryland 20852.**

*IGEN is a biotechnology company located in Maryland close to Washington, DC, specializing in the development of a new generic diagnostic technology and in the pharmaceutical and therapeutic applications of catalytic antibodies (ABZYMES™).*

(NW3557)A

EEOC



University of Zürich  
Faculty of Medicine and  
Faculty of Natural Sciences

## FACULTY POSITION IN BIOCHEMISTRY

Applications are invited for a tenured faculty position (associate professorship, Extraordinariat) at the Department of Biochemistry. Candidates are expected to have a creative research programme in protein biochemistry and a genuine interest in the teaching of biochemistry to medical and science students.

Applications including curriculum vitae, bibliography synopsis of past and planned future research, and a record of teaching experience should be sent to the Chairman of the Search Committee, Professor Christian Bauer Physiologisches Institut der Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland.

Deadline June 15, 1989.

(W6022)A

## CHAIR DEPARTMENT OF VETERINARY BIOLOGY College of Veterinary Medicine University of Minnesota

Applications and nominations are invited for a tenured Professor and Chair of the Department of Veterinary Biology. The department includes 17 faculty in the disciplines of anatomy, biochemistry, physiology and pharmacology, and occupies modern laboratory and teaching facilities. The College is located on the Twin Cities Campus in St. Paul with the opportunity for interaction with colleagues from many other units of the University including the Medical School, the College of Biological Sciences and the College of Agriculture. Our location also provides the cultural advantages of a large metropolitan area together with proximity to a wide variety of outdoor activities.

Candidates must have the Ph.D. degree or relevant foreign equivalent and preference will be given to individuals who also hold the D.V.M. degree or foreign equivalent. The appointee will be expected to have a distinguished record of research and teaching accomplishments, with proven leadership and administrative abilities. The appointee must be able to: 1) provide leadership, both in professional and graduate education, and in developing competitive research programs, and 2) have an understanding of future trends in the veterinary profession and current issues relating to veterinary medical education. Letters of application should include a current curriculum vitae and a statement of goals, plus names and addresses of at least 3 persons who will provide letters of reference.

Applications with supporting material will be accepted until June 15, 1989, and should be sent to **Dr. Alice A. Larson, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55108.**

*The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities.*

(NW3547)A

## Post Doctoral Research at The Wistar Institute

Post doctoral positions are available at The Wistar Institute for candidates with a strong background in molecular and cell biology, virology, immunology, genetics or protein chemistry. Specific areas of research include: human retroviruses; DNA/RNA tumor viruses; growth factors; oncogenes; pattern formation in embryos; molecular mechanisms involved in the immune response; x-ray crystallography of virus receptor interaction; and aging in tissue culture. The Wistar Institute is an independent institution, located on the campus of the University of Pennsylvania and is equipped with the latest biotechnology facilities.

Starting salary at \$20,000 a year. Interested applicants are requested to send a curriculum vitae and the names of 3 references to: **Coordinator of Research Training, THE WISTAR INSTITUTE, 36th and Spruce Streets, Philadelphia, PA 19104.** Equal opportunity employer, M/F.

THE  
WISTAR  
INSTITUTE



(NW3540)A

## MEDICINAL CHEMIST Peptide Design & Synthesis

COMMITTED  
TO  
SCIENTIFIC  
EXCELLENCE



The Sterling Research Group, the worldwide pharmaceutical research and development organization of Sterling Drug Inc., announces an opening for a Medicinal/Organic Chemist in the Medicinal Chemistry Department of its Rensselaer, New York facility.

An excellent opportunity exists for an organic chemist who has experience in the synthesis of peptides to join an expanding group committed to the discovery of peptides and peptide mimetics as potential therapeutics. Experience in solid-phase peptide synthesis would be an advantage.

The successful candidate will have a doctoral degree in chemistry or a related discipline.

Sterling offers competitive salaries and a comprehensive benefits program, along with outstanding career opportunities for personal and professional growth. For confidential consideration, please forward your resume with salary history/requirements to:

**Jo Ann Fitzgerald**  
Employee Relations Administrator

## STERLING RESEARCH GROUP

A Division of Sterling Drug Inc.  
81 Columbia Turnpike  
Rensselaer, NY 12144

A Subsidiary of the  
**EASTMAN KODAK COMPANY**

Equal Opportunity Employer M/F/H/V

(NW3542)A

## DEPARTMENT OF PSYCHIATRY UNIVERSITY OF TORONTO

### Applications are invited for the position of ASSISTANT PROFESSOR

in the area of Molecular Neurogenetics as applied to psychiatric disorders. This position requires a Ph.D. or other doctoral degree and significant post-doctoral experience in molecular genetics, molecular neurobiology or an equivalent field. The successful candidate will be expected to work collaboratively with a multidisciplinary neuroscience/neuropsychiatric research group comprised of basic and clinical researchers investigating the neurobiology and neurogenetics of psychiatric disorders. The position requires demonstrated ability to conduct independent research within a collaborative group and to compete for extramural funding.

The position is tenure track equivalent supported initially by the I'Anson fund. Salary is commensurate with rank and experience. The position also includes a one time start up research grant.

The University encourages both men and women to apply for positions. In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada.

Candidates should submit a letter of application, a curriculum vitae and the names of three referees from whom letters of recommendation may be solicited. Please submit responses to: **Dr. Jerry I. Warsh, Director of Research, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario M5T 1R8.**

The successful candidate would be expected to take up the appointment prior to September 1, 1989.

**Closing date for applications is May 19, 1989.** (NW3559)A



## NATIONAL UNIVERSITY OF SINGAPORE INSTITUTE OF MOLECULAR AND CELL BIOLOGY

### POST-DOCTORAL APPOINTMENT IN HYBRIDOMA TECHNOLOGY

The Institute of Molecular and Cell Biology invites applications from candidates who have expertise in hybridoma technology.

The Institute, which was recently established to undertake basic biomedical and biotechnological research, has three principal research areas related to Cell Regulation, Infectious and Genetic Diseases and Molecular and Cell Biology of Plants. The Institute, which is adjacent to the University's biomedical complex and the National University Hospital, has 19 laboratory modules of 500 square feet each.

The successful candidate should have post-doctoral experience in the field of hybridoma technology. He/She will be required to provide a service for generating monoclonal antibodies and also undertake an independent research programme.

Salary will be commensurate with experience; leave, medical and other benefits will be provided. Applicants should send their curriculum vitae and names and addresses of 3 referees to the:

**Director  
Personnel Department  
National University of Singapore  
10 Kent Ridge Crescent  
Singapore 0511**

Enquiries may also be sent through BITNET to:

**PERSDEPT @ NUSVM**

(W6034)A





Department of Scientific and  
Industrial Research



## SCIENTIST- CYTOGENETICIST

### Animal Gene Mapping Programme Biotechnology Division

The DSIR is the largest scientific research organisation in New Zealand. Three Divisions (Grasslands, Biotechnology and Plant Physiology) are based in the Palmerston North Centre.

Palmerston North (population 70,000), is located in a pleasant rural area, about three quarters of an hour's drive from the coast. The city caters for a wide range of recreational activities and has excellent educational facilities.

The Animal Gene Mapping programme is an expanding group involved in mapping the genomes of sheep, cattle and horses in the Animal Gene Technology programme.

A permanent position exists for a Cytogeneticist with experience in high resolution banding, in-situ hybridisation, and comparative karyotyping of mammalian chromosomes.

The person will be actively involved in collaborating with molecular geneticists in constructing a physical map of the sheep genome.

Applicants should possess a Ph.D in genetics, biochemistry, physiology or zoology and have suitable post-graduate experience in human, or preferably, animal cytogenetics.

The ability to work effectively in a multi-disciplinary group situation is essential.

Salaries will be commensurate with qualifications and experience. Relocation expenses are negotiable. Promotion and career advancement are dependent on performance.

Please apply in writing quoting reference No. BIO 18, enclosing a curriculum vitae and the names of at least two referees by April 27, to:

The Personnel Officer,  
DSIR, Private Bag  
Palmerston North

Fax: (063) 61-130, Phone: (063) 68-019

An equal opportunity employer.

(W6023)A

# nature

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## THE AUSTRALIAN NATIONAL UNIVERSITY

POSTDOCTORAL FELLOW/  
RESEARCH FELLOW, DIVISION OF  
CLINICAL SCIENCES

The John Curtin School of  
Medical Research

The colon cancer group seeks an experienced molecular biologist to join its study of colon epithelial transformation, invasion and metastasis. The successful applicant will contribute to an established project researching the genetic basis of colon cancer at a molecular level, including the defective alleles identified in hereditary and sporadic forms of colon cancers. The laboratories are fully equipped for molecular biology.

Enquiries may be directed to: Dr N.G. Ardlie, Acting Head of the Division of Clinical Sciences (062) 492885, The John Curtin School of Medical Research. Closing date: 5 May 1989. Ref: JC17.3.1.

Salary: Research Fellow; A\$30,737-A\$40,100 p.a. Postdoctoral Fellow Grade 1 (fixed point): A\$26,617-A\$30,360 p.a. Appointment: Research Fellow up to three years, possibility of extension to five years; Postdoctoral Fellow normally two years, possibility of extension to three years. Applications should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information may be obtained from the Registrar or from Appointments (36191), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

The University is an Equal Opportunity Employer (W6025)A

### UNIVERSITY OF DUNDEE DEPARTMENT OF BIOLOGICAL SCIENCES RESEARCH ASSISTANTS IN PLANT MOLECULAR BIOLOGY

Applications are invited for the position of Postdoctoral Research Assistant (1A) and Postgraduate Research Assistant (1B) to join a group studying the splicing of pre-mRNAs in plants. The programme is AFRC-funded for 3 years and will involve the isolation of plant UsnRNA genes and the study of their genetic organisation and expression.

The postdoctoral RA will be appointed on the 1A scale (starting salary (£9,865-£11,070) and the postgraduate RA on the 1B scale (£8,675).

Further information is available from Dr John W S Brown, Tel. 0382-23181 Ext. 4278.

Applications (CV and names of 2 referees) to the Personnel Office, The University, Dundee, DD1 4HN, quoting Ref: EST/412/89/N. Closing Date: 30 April 1989.

(8891)A

## OXFORD UNIVERSITY NUFFIELD DEPARTMENT OF PATHOLOGY

### Project on the Spread of Cancer in the Human Body Research Assistant Grade 1A

Applications are invited from postdoctoral scientists for a responsible position in a team studying basic cellular and molecular mechanisms by which malignant tumours spread (metastasis) to distant organs in the body. The challenge of the job is that it focuses directly on the major problem of cancer: namely the formation of multiple scattered secondary tumours and how to control their growth. Experience in cell biology, biochemistry/molecular biology/virology or genetics will be an advantage but not essential, if the candidate has other relevant practical expertise.

Starting salary up to a maximum of £14,500 on the University Research Staff 1A scale (£9,815,720), depending on age and experience. The project is financed for 3 years by the Cancer Research Campaign and the post is vacant.

Further information may be obtained from: Dr. D. Tarin, Nuffield Reader in Pathology, Nuffield Department of Pathology, John Radcliffe Hospital, Headington, Oxford OX3 9DU. (Telephone (Oxford) (0865) 817797) to whom applications with full c.v. and names of 2 referees should be submitted by 27/4/89.

Oxford University is an equal opportunity employer.

(8886)A

## POSTDOCTORAL POSITIONS

Three postdoctoral positions available immediately in the Laboratory of Viral Diseases, NIAID. Dr. Bernard Moss, Laboratory Chief. Studies include 1) Biosynthesis, assembly and transport of HIV proteins using monoclonal antibodies (Dr. Jonathan Yewdell, see Cell 52: 843, 1988) and 2) CD4 gp120 interactions and the design of CD4-based targeted AIDS therapeutics (Dr. Edward Berger, see Nature, 335: 369, 1988). Experience in hybridoma technology, protein chemistry, genetic engineering, or HIV virology preferred but not required. Starting salary commensurate with experience. Curriculum vitae and four letters of reference to:

Dr. Jonathan Yewdell  
NIAID, Twinbrook II  
12441 Parklawn Drive  
Rockville, MD 20852

or

Dr. Edward Berger  
NIAID, NIH  
Bldg. 4, Room 232  
9000 Rockville Pike  
Bethesda, MD 20892

NIH IS AN EQUAL OPPORTUNITY EMPLOYER



## CITRUS HORTICULTURIST

Academic career-track, 11-month Assistant or Associate Horticulturist in Integrated Citrus Management to develop more efficient cultural practices for citrus production, especially in the areas of nutrition and irrigation. The position is a 75% Agricultural Experiment Station research appointment with a 25% appointment in Cooperative Extension within the Department of Botany and Plant Sciences at the University of California, Riverside. Responsibilities include development of a research program to adapt and test the efficacy of major advances in basic research to the production of citrus. Field research will be conducted at appropriate off-campus locations, including the San Joaquin Valley.

Extension responsibilities include conducting training sessions and short courses to disseminate research results to the industry and to facilitate their implementation by growers. The position is intended to combine aspects of both applied and basic research. Requires a Ph.D. degree specializing in Plant Physiology, Botany, Horticulture, or other plant science with demonstrated experience in designing, conducting, analyzing, and interpreting field research, especially the physiology of plant nutrition or irrigation of tree crops.

Send letter of application, curriculum vitae, statement of research interest, transcripts (recent graduates), and at least three confidential letters of evaluation to: **Dr. Carol J. Lovatt, Chair, Search Committee, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.** The application deadline is May 31, 1989.

*The University of California, Riverside, is an Affirmative Action/Equal Opportunity Employer. Minorities and women are encouraged to apply.* (NW3546)A

### UNIVERSITY OF CAMBRIDGE

#### Department of Biochemistry

#### POSTGRADUATE AND POSTDOCTORAL RESEARCH ON SUGAR TRANSPORT PROTEINS

Certain bacterial sugar transport proteins are homologous to the mammalian glucose transporters (*Nature*. 325, 641, 1987; *J. Biol. Chem.* 263, 8003, 1988; *Ann. Rev. Physiol.* 51, 459-471, 1989). Their molecular architecture and mechanism are being elucidated by a combination of protein chemistry, mutagenesis, recombinant DNA techniques, gene sequencing, etc. Facilities for automated nucleotide synthesis, peptide synthesis and protein sequencing are available.

Applicants for a postdoctoral research post (age-related scales £12,415 - £14,500) or Ph.D. studentships may be a qualified in Biochemistry, Genetics, Microbiology or allied subjects. Send full C.V. plus names of two referees to **Dr Peter Henderson, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW.** Tel: (0223) 333622. (8898)A

### JUNIOR FACULTY POSITION

available in the pharmacology and biochemistry of antineoplastic agents targeted against specific human cancer types. Studies will involve the modulation of anticancer agents with thymidylate synthase as the primary target. The focus will be on metabolism of reduced folates, modulation of reduced folate pools, and optimization of drug scheduling for therapeutic gain. Research will also identify factors that influence the regulation and turnover of thymidylate synthase in elucidating the importance of the enzyme as a therapeutic target. Candidates should have the Ph.D. degree with a minimum of 3 years postdoctoral experience. A strong background in Biochemistry is advantageous. Send curriculum vitae and names of 3 references to:

**Dr. J. A. Houghton,  
Laboratories for Developmental Therapeutics,  
Department of Pharmacology,  
St. Jude Children's Research Hospital,  
332 North Lauderdale, Memphis, TN 38101.**

*Equal Opportunity/Affirmative Action Employer.* (NW3556)A

*The Polytechnic operates a range of degree and post graduate level courses, has a vigorous and successful record of consultancy and research. Due to Corporate Status the Polytechnic has entered a new and challenging phase of its development. To maintain and extend the programme of work, applicants are invited for the following post:*

### FACULTY OF SCIENCE

## Head of School of Pharmaceutical and Chemical Sciences

£24,084 - £26,538

Applicants for the post should possess both high academic credibility and the ability to manage and lead the School as a budget centre.

The School of Pharmaceutical and Chemical Sciences is one of three Schools in the Faculty of Science and covers the disciplines of pharmacy (except for pharmacology) and of chemistry. The School has an establishment of over 30 academic staff.

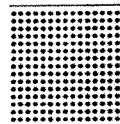
The person appointed would be eligible for consideration for appointment to the Professoriate.

This is a readvertisement; applications from candidates responding to the previous advertisement for the post will be taken into consideration — there is no need for a re-application.

**Further details and application forms are available from Personnel Services, Langham Tower, Ryhope Road, Sunderland SR2 7EE, or telephone (091) 5152429. Informal enquiries may be made to Professor J. R. Brown, Dean of Faculty, telephone 091 5152504.**

**Closing date: 30th April, 1989.**

(8941)A



## SUNDERLAND POLYTECHNIC

### UNIVERSITY OF EDINBURGH

#### Department of Preclinical Veterinary Sciences

### LECTURESHIPS

Applications are invited for two Lectureships in the above Department which has teaching and research commitments in Anatomy, Biochemistry, Pharmacology and Physiology.

**Lectureship in Systems Physiology.** This post will be funded under the terms of the New Academic Appointments Scheme and will be available from October 1989 subject to UFC approval. The holder will be expected to pursue research in some branch of mammalian systems physiology and encouraged to seek external funding for research. Systems of particular interest to the Department are the cardiovascular and respiratory systems but applicants with research interests in other fields of mammalian physiology will also be considered. The successful applicant would be expected to participate in the undergraduate teaching and postgraduate training programmes of the Department. The initial salary will be on the Lecturer A scale (£9,260-£14,500 p.a.) or exceptionally on the Lecturer B scale (£15,105-£19,310 p.a.) Ref. No. 1425

**Lecturer in Biochemistry.** This post is tenable from October 1989 and arises from the retirement of a member of staff. It is a joint appointment held in the Departments of Preclinical Veterinary Sciences and Biochemistry. Candidates should be active in any aspect of biochemistry, cell biology or molecular biology relevant to veterinary science. The successful applicant would be expected to participate in undergraduate teaching and postgraduate training programmes of both departments and encouraged to seek external funding.

Salary will be on the Lecturer Scale A (currently £9,260-£14,500 p.a.) Ref. No. 1426 Applications (six copies) including a curriculum vitae and the names and addresses of two referees, should be submitted in writing to **The Secretary to the University, Old College, South Bridge, Edinburgh, EH8 9YL.** The closing date for applications is 1st May 1989. The appropriate reference number should be quoted in the application. Enquiries, which are welcomed, should be directed to Professor C. R. House, Department of Preclinical Veterinary Sciences, University of Edinburgh, Summerhall, Edinburgh, EH9 1QH (telephone number, 031-667-1011, Ext. 5344). (8910)A

**MRC PROTEIN PHOSPHORYLATION GROUP  
BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF DUNDEE**

**POSTDOCTORAL  
POSITIONS**

Following expansion of the research programme by the Medical Research Council, several postdoctoral positions have become available to work with Professor Philip Cohen and Dr Tricia Cohen. The work is interdisciplinary and applications are invited from candidates with experience in any of the following areas; molecular genetics, protein chemistry, enzymology, molecular pharmacology and immunocytochemistry. A major aspect of the research is concerned with the structure and regulation of protein phosphatases. Recent work within the Group has demonstrated that these enzymes play key roles hormonal regulation in cell division and in tumour suppression, and several new phosphatase genes have been isolated. Novel mechanisms for controlling their activities have also been identified that involve interaction with specific targeting subunits. The positions are available from October 1989, but candidates wishing to start at any time up to Spring 1990 will be considered. The appointments are for three or five years in the first instance and will be made at an appropriate point on the University Grade 1A scale, depending on qualifications and experience.

The Department is one of the most highly funded in Britain and offers outstanding research facilities and working environment. Housing and accommodation costs are among the lowest in the UK and access to outdoor recreational facilities is unrivalled.

Applications containing a full curriculum vitae and the names and addresses of three referees should be sent to **Professor Philip Cohen, FRS, FRSE, Department of Biochemistry, The University, Dundee DD1 4HN, Tayside, Scotland** as soon as possible. Informal enquiries by telephone (0382-23181) to either Philip Cohen (ext 4238, 4241) or Tricia Cohen (ext 4240). (8901)A

**UNIVERSITY OF GLASGOW  
ORAL BIOLOGY GROUP  
POST-DOCTORAL (BIOCHEMISTRY)  
RESEARCH ASSISTANT**

Applications are invited from suitably qualified candidates for the post of Research Assistant for a Biochemistry (Range 1A) project in the Dental Hospital and School Oral Biology Group.

Applicants must have a good honours degree in Biochemistry or an allied subject and possess (or expect to possess shortly) a Ph.D. in this discipline.

The Group has well equipped laboratories and the work will involve the analysis of dietary carbohydrate in saliva and dental plaque by HPLC. The project is supported by a research contract with Mars Confectionery. The appointment will be for a period of one year with the possibility of renewal of contract for a further year.

Starting Salary — £9,865 (under review) including U.S.S.

Appointment to commence immediately.

Applications, together with a C.V. and the names of two academic referees should be sent to:

**Dr D.A.M. Geddes and Dr J.A. Beeley,  
Oral Biology Group,  
Glasgow Dental Hospital & School,  
378 Sauchiehall Street, Glasgow, G2 3JZ**

from whom further particulars may be obtained.

Closing date: 21st April, 1989.

(8904)A

**UNIVERSITY OF ABERDEEN  
DEPARTMENT OF BIO-MEDICAL PHYSICS AND BIO-ENGINEERING**

**RESEARCH FELLOW**

The successful applicant will work on a joint project with the Department of Ophthalmology on the potential of digital techniques in retinal imaging. The post-holder will work closely with ophthalmologists and will be responsible for developing software for image analysis and the assisting with the design of optical systems. Ability to program in C is essential and familiarity with optical design would be an advantage. The post is tenable for one year, within the range £9,865-£12,760 on the Research and Analogous Staff Scale 1A, depending upon qualifications and experience.

Informal enquiries to Dr P Sharp on 0224 681818 ext 53209. Further particulars and application forms from **The Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX**, to whom applications (2 copies) should be returned by 28 April 1989 quoting reference number LW/014. (8913)A

**THE AUSTRALIAN  
NATIONAL UNIVERSITY  
POSTDOCTORAL FELLOW/  
RESEARCH FELLOW IN  
VISUAL  
ELECTROPHYSIOLOGY**

Centre for Information Science Research

Applications are invited from suitably qualified electrophysiologists with experience in intracellular electrophysiology, dye injection and computer-aided data collection and analysis, to work with a team in the Centre for Visual Sciences, investigating visual processing in insects. A PhD is essential, and experience in investigating processing at higher levels of the visual pathway is highly desirable. This post is funded by the Centre for Information Science Research and it is expected that the successful applicant will be involved in the Centre's activities.

For further information contact Dr M. W. Srinivasan (tel. [010-61] (062) 492409, Fax No. 489995, Telex No. [AA] 62219.)

Closing date: 30 April 1989 Ref: CIS 22.3.1

**SALARY:** Research Fellow: A\$30,737-A\$40,100 p.a.; Post-doctoral Fellow Grade 1 (fixed point); A\$26,617-A\$30,360 p.a. **APPOINTMENT:** Research Fellow up to three years, possibility of extension to five years; Postdoctoral Fellow normally two years, possibility of extension to three years. **APPLICATIONS** should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from Appointments (36192), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

**THE UNIVERSITY IS AN  
EQUAL OPPORTUNITY  
EMPLOYER**  
(W6024)A

**ANATOMIST**

**The Department of Anatomy,  
University of Saskatchewan,  
College of Medicine,**

invites applications for a faculty appointment at the rank of Assistant Professor. Applicants should have post-doctoral experience with a strong research program in any area of anatomical sciences; however, research in functional anatomy is preferred. The successful candidate will be responsible for teaching gross and neuroanatomy to Physical Therapy students. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

Application, including curriculum vitae, names of three referees, and an outline of proposed research and previous teaching experience should be submitted to: **Dr. G. D. Burkholder, Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0.** (NW3550)A

**PROTEIN CHEMIST**

Successful applicant will purify, analyze and identify proteins, will study physico-chemical properties of proteins by spectroscopy, calorimetric, electrophoretic and chromatographic methods, will conduct research to determine interactions between proteins and non-protein compounds. Minimum requirements. Master Degree in biochemistry or related field with one year experience as a protein chemist and one year experience with computer analysis of protein structure and with multicomponent analysis of derivative spectra of proteins. Salary \$18,852 year, 40 hours a week, 8 am - 5 pm. Please apply to: **Wyoming State Employment Service, 112 South 5th Street, Laramie, Wyoming 82070.** Refer to Job Order #WYO-201311. N(W3570)A

**UNIVERSITY OF CAMBRIDGE  
DEPARTMENT OF BIOCHEMISTRY  
SENIOR TECHNICAL OFFICER**

Senior Technical Officer to take up appointment on 1 October 1989. Applications invited from experienced NMR spectroscopists. The successful candidate will be expected to oversee the high-field NMR facilities located within the Department.

Salary on scale £13,365-£19,310.

Further information from **Secretary of Appointments Committee for Senior Technical Officers, General Board Office, The Old Schools, Cambridge CB2 1TT.** Applications (10 copies) with curriculum vitae and names of three referees by 26 April 1989. (8897)A





Public Service Commission  
of Canada

Commission de la fonction  
publique du Canada

## ***Biologists (BI-04)***

Health and Welfare Canada  
Health Protection Branch  
Ottawa, Ontario

The Bureau of Biologics requires two experienced scientific professionals to head two different areas within the Bureau.

### ***Bacterial Antigens and Antisera Section***

***Reference number: S-88-31-8119-47JG-G26***

As a Biologist with the Bureau, you will direct the activities of this Section. This involves regulatory control testing and evaluation of the safety and efficacy of various bacterial vaccines and antisera, as well as other biological drugs prepared with biotechnology for human use in the prevention and treatment of diseases. You will also direct and coordinate development research programs on the above-mentioned drugs.

Requirements include graduation with a doctoral degree from a recognized university in a biological science or a lesser degree with evidence of research experience. You must also possess laboratory and research experience in microbiology, molecular biology and immunology. Knowledge of English is essential.

### ***Immunology Section***

***Reference number: S-89-31-5221-47JG-G26***

As a Biologist with the Bureau, you will oversee the activities of this section. Such a responsibility involves regulatory control testing and evaluation of the safety and efficacy of various biological drugs (hormones, enzymes, allergenic products) prepared by different production methods including biotechnology. We will rely on you to act as a consultant in the appraisal and evaluation of new drug submissions and to evaluate operating procedures of Canadian and foreign pharmaceutical manufacturers. You will also direct and coordinate development research programs on the above-mentioned drugs.

Education includes graduation with a doctoral degree from a recognized university in a biological science or a lesser degree with evidence of research experience. You must also possess laboratory and research experience in biochemistry and immunology. Experience in molecular biology and microbiology would be a definite asset. Knowledge of English is essential.

***We offer*** a salary ranging from \$48,139 to \$55,591 commensurate with your qualifications and experience.

Please forward your résumé and/or application form, quoting the appropriate reference number to: ***Joan Girling, (613) 996-8054, Public Service Commission of Canada, National Capital Region, 171 Slater Street, Ottawa, Ontario K1A 0M7.***

***Closing Date:*** 31 May 1989

# Canada

W(6036)A

Public Service Commission of Canada is an equal opportunity employer

## INSTITUTE OF BIOTECHNOLOGY, UNIVERSITY OF HELSINKI RESEARCH DIRECTORS

The Institute of Biotechnology, University of Helsinki, is a new inter-faculty research unit with a special Ph.D. training program. At present, about 50 persons are working at the Institute, which has an annual budget of about USD 2.5 million.

The Institute will focus on basic research in different fields of molecular biology essential for the development of modern biotechnology. The research will be carried out in programs led by the Director of the Institute and Research Directors, who have the title of Professor.

The Institute has modern facilities for molecular and cell biology. It has and will have funds available for special equipment needed in new research programs, such as X-ray crystallography.

Applications are invited for research directors in the general field of molecular biology, preferably from those with training in:

- **Plant Molecular Biology**
- **Microbiology**
- **Biological Structures (Crystallography, Electron Microscopy)**
- **Developmental Genetics**

The research directors are appointed for a 5-year period (renewable) but can be made permanent for special reasons. The applicants should hold a Ph.D. or an equivalent degree, a substantial publication record and experience in directing scientific research. The salary is at the level of university professor in Finland (FIM 175,000-225,000; equivalent to USD 41,000-52,000).

Applications should be addressed to the **Board of the Institute of Biotechnology, Registrars Office of the University of Helsinki, Hallituskatu 8, SF-00100 Helsinki, Finland.** The applications should be at the University of Helsinki not later than May 31st, 1989.

Applications should include:

- 1) **curriculum vitae**
- 2) **list of publications 1980-1989**
- 3) **reprints of publications 1985-1989**
- 4) **a 5-year research plan**, with an estimation of the personnel required and possible plans for financing
- 5) **date when the applicant is available**

The Research Directors will be appointed by the Chancellor of the University after consultation with the Scientific Advisory Board of the Institute. The positions will be filled during the autumn of 1989.

For further information please contact Prof. Lauri Saxen (Chairman of the Board; telephone +358 0 4346433) or Prof. Helge Gyllenberg (Acting Director; telephone +358 0 4346041 or telefax +358 0 4346028).

W(6031)A

## THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

### POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post, commencing as soon as possible, for a fixed term of 36 months, to study the actions and metabolism of extracellular ATP in human articular cartilage.

The project is funded by a grant from the Wellcome Trust to Dr A M Caswell and Professor R G G Russell (Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School).

Applicants should have a PhD or equivalent relevant experience. Expertise in tissue culture techniques advantageous.

Salary on the RA IA scale (£9865-£11070) according to qualifications and relevant experience.

Informal enquiries may be made to Dr A M Caswell (tel (0532) 333114).

Application forms and further particulars may be obtained from and completed applications forwarded to the **Registrar, the University, Leeds LS2 9TJ** (tel (0532) 333963 — direct line), quoting reference number 83/94.

Closing date for applications\* 27 April 1989. (8933)A

## THE AFRC INSTITUTE OF HORTICULTURAL RESEARCH

EAST MALLING

Scientific Officer (Band II/III)

A Scientific Officer is required in the Plant Physiology Department to assist the Head of Department in the development of existing biochemical research programmes, especially on plant hormone receptors.

Qualifications: Honours degree or equivalent in Biological Sciences with relevant post-qualifying experience (Band II); Ordinary Degree, HNC, HND or equivalent (Band III). Working experience with modern biochemical techniques an advantage.

Salary: In range £8,574-£10,994.

Further details and application form from the Personnel Officer, Bradbourne House, East Malling, Maidstone, Kent ME19 6BJ quoting Ref. 57/EM. Closing date 27th April 1989.

The Institute of Horticultural Research is an Equal Opportunities Employer. (8925)A

## MOLECULAR GENETICIST

### LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

The Lady Davis Institute, a research department of the Sir Mortimer B. Davis-Jewish General Hospital, is seeking a molecular geneticist at the assistant/associate professor level. Applicants should hold Ph.D. and/or M.D. degree(s), have significant post-doctoral experience and the potential for developing a vigorous independent research program on gene expression at a locus involved in human disease. Preference will be given to candidates with M.D. degrees who are qualified and willing to spend 20% of their time in the Division of Genetics of the Department of Medicine.

Send curriculum vitae, statement of research goals and the names of three references to:

**Dr. Herbert M. Schulman, Acting Director**  
**Lady Davis Institute for Medical Research**  
**Sir Mortimer B. Davis-Jewish General Hospital**  
**Montreal, Quebec, Canada H3T 1E2**

(NW3549)A

## RESEARCH ASSISTANT PROFESSOR

There is an immediate opening at the level of Research Assistant Professor in the Department of Physiology, University of Florida, College of Medicine. Applicants should have expertise in the fields of cellular and molecular neuroendocrinology. They should be willing to develop their independent research programs and interact with existing faculty members in the areas of the molecular biology of angiotensin II and/or growth factors in the brain. Ph.D. and/or M.D. is required and postdoctoral experience would be advantageous, though is not essential. The salary shall be \$25,000.00-\$32,000.00 p.a., depending upon experience. Applications, including a C.V., a summary of research experience and the names and addresses of three (3) references should be sent to:

**Dr. Colin Sumners, Department of Physiology, University of Florida, College of Medicine, Box J-274, JHMC, Gainesville, FL 32610, USA**

(NW3554)A

## VIROLOGIST

A Virologist with an interest in antiviral research is being sought to play a major role in two funded research grants. The candidate will explore the active components of natural products found to be effective in vivo for their effect on the replication cycle and pathogenesis of hepadnaviruses. The successful candidate should have some knowledge of the molecular biology of hepadnaviruses, tissue culture techniques and animal models useful for the study of hepadnaviruses. Although the project is funded, the candidate, in time, will be encouraged to seek independent funding to pursue related research and become eligible for a tenure track position.

Please send curriculum vitae and letters of reference to: **Virologist Search Committee, c/o Personnel Dept., Fox Chase Cancer Center, 7701 Burholme Ave., Phil., PA 19111.**

*We are an Equal Opportunity Employer* (NW3564)A

## UNIVERSITY OF ST ANDREWS

Department of Biology and Preclinical Medicine

### LECTURESHIP IN BIOLOGY

Applications are invited for the above post from candidates with a strong research record in any area of Plant Biology. Candidates interested in Plant Molecular Biology and/or Pathology will be welcome provided that they have a broad background in Plant Biology.

The appointment is tenable from 1st October 1989 and the salary will be on either the Lecturer Grade A scale £9,260 to £14,500, or Grade B scale £15,105 to £19,310 per annum, initial appointment probably on the Grade A scale.

Further particulars and application forms are available from the **Director of Personnel Services, The University, College Gate, St Andrews, Fife KY16 9ANJ, to whom applications should be submitted not later than 25th April 1989.** (8906)A

# Research Scientist/Immunology FRANCE

**ROUSSEL-UCLAF** is a major pharmaceutical company involved in Chemistry and Biotechnology for pharmaceutical, veterinary and agricultural activities.

The Company is seeking an immunologist for its Biotechnology Department located in suburban Paris. The successful candidate will join a multidisciplinary group, and head a team of 2/3 technicians. The research programs of the group are focused on chemicals and cytokines with immunomodulatory properties.

Requirements include a Ph.D and 2/3 years relevant post-doctoral : international experience would be appreciated. Enthusiasm and communication skills are important qualities. A substantial experience in cellular Immunology will be determinant.

Send resume, list of publications and the names of three references in confidence n° 107/N to Claudine MAUSHART, 35, boulevard des Invalides, 75007 PARIS (FRANCE).

**ROUSSEL UCLAF** 

(W6018)A

CURRICULUM

## CENTRAL BIRMINGHAM HEALTH AUTHORITY RESEARCH ASSOCIATE (Clinical Chemistry)

Based in the Cell Culture/Tissue Laboratory, you will provide a service to the Molecular Biology and Immunoassay sections of the Wolfson Research Laboratories. Cell/Tissue Culture is a relatively new area of expertise and you will be expected to help run a rapidly changing, expanding and important section within the laboratories. Opportunities may exist for the successful candidate to obtain further qualification.

Application form and job description from **Personnel Department, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH** telephone 021 472 1311 ext 3003.

Additional information and informal visits may be arranged with the laboratory Manager, Dr. Wendy Ratcliffe ext 4560. Closing date 21.4.89.

*The Authority is Committed to Equal Opportunities in Employment.* (8927)A

## OXFORD UNIVERSITY NUFFIELD DEPARTMENT OF PATHOLOGY

### Project on the Spread of Cancer in the Human Body Research Assistant Grade 1B

Applications are invited from graduates with a good honours degree for a training position in a team studying basic cellular and molecular mechanisms by which malignant tumours spread (metastasise) to distant organs in the body. The challenge of the job is that it focuses directly on the major problem of cancer: namely the formation of multiple scattered secondary tumours and how to control their growth. Experience in cell biology, biochemistry/molecular biology/virology or genetics will be an advantage but not essential, if the candidate has other relevant practical expertise.

Starting salary up to a maximum of £11,070 on the University Research Staff 1B scale (£8,675-£13,365), depending on age and experience. The project is financed for 3 years by the Cancer Research Campaign and the post is vacant.

Further information may be obtained from:

**Dr. D. Tarin Nuffield Reader in Pathology Nuffield Department of Pathology John Radcliffe Hospital Headington, Oxford OX3 9DU**

[Telephone: Oxford (0865) 817797]

to whom applications with full c.v. and names of 2 referees should be submitted by 27-4-89.

*Oxford University is an equal opportunity employer.* (8888)A

## PROTEIN

### CRYSTALLOGRAPHER

Postdoctoral position open for cooperation on the X-ray analysis of photosystem I. Crystals diffract to 4Å (to be improved), facilities include area detector, rotating anodes, graphical displays, Vax computers.

Send c.v. to **Prof. W. Saenger, Institut für Kristallographie der Freien Universität, Takustr. 6, D-1000 Berlin 33 (FRG).** (W6019)A

## Biochemical and Electrophysiological Pharmacologists

Medicinal Research Centre  
Harlow, Essex

Beecham, one of the UK's leading pharmaceutical companies, has embarked on a major expansion of CNS research.

Consequently, we now have excellent opportunities for a high calibre CNS biochemical-pharmacologist and an electrophysiologist to work as part of large, multi-disciplinary project teams. We are particularly interested in individuals who hold a PhD and have had several years' experience of CNS electrophysiological and/or biochemical techniques.

However, we should also like to hear from people, both at PhD and BSc level, who have less experience, but a keen interest in CNS electrophysiological and biochemical techniques.

We offer excellent career prospects, a progressive salary structure which recognises personal achievement, and the comprehensive range of benefits associated with a large company: including a non-contributory pension, free life assurance, company bonus and flexible working hours. Relocation assistance will be provided, where appropriate.

For an application form, please telephone: Harlow (0279) 622022 during office hours, or our 24-hour answering service on (0279) 622030. Alternatively, send your full CV, indicating your present salary level and quoting reference 20/89 to the Personnel Manager, Beecham Pharmaceuticals Research Division, Medicinal Research Centre, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD.

(8939)A

**Beecham**  
Pharmaceuticals





## DEAN GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

As part of a plan to enhance its effectiveness and impact as a major research center, The University of Medicine and Dentistry of New Jersey seeks an accomplished Scientist as Dean of its Graduate School of Biomedical Sciences. The Graduate School consists of 14 departments on two campuses offering programs leading to the Ph.D., M.D./Ph.D. and D.M.D./Ph.D.

The successful candidate will have a Doctoral degree in an appropriate discipline plus a track record of substantial research activity sufficient for appointment as a tenured Professor. Administrative experience is desirable.

Review of applications will begin immediately. The University will continue to accept applications until the position is filled. Applicants should send a complete curriculum vitae and a brief letter of interest to: **Harvey Ozer, M.D., Search Committee Chair, Chairman, Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, (N), 185 South Orange Avenue, Newark, NJ 07103-2757.**

The UMDNJ is an Affirmative Action/Equal Employment Opportunity Employer M/F/H/V.



(NW3574)A

## UNIVERSITY OF BIRMINGHAM FACULTY OF MEDICINE AND DENTISTRY RESEARCH ASSOCIATE IN MOLECULAR MECHANISMS OF THYROID HORMONE ACTION

Applications are invited for the post which has been funded for 2 years. The appointee would join an established programme of molecular biology in endocrinology within the Department of Medicine. The post specifically involves the study of molecular mechanisms of thyroid hormone action; techniques to be used include cell culture, plasmid preparation and cDNA/mRNA hybridisation assays. Applicants should be graduates in a biological subject. Previous experience in molecular biology is not essential.

Starting date: July 1989 or as soon as possible thereafter. Salary on the Research Associate scale £8,675 – £11,680 plus superannuation. Informal enquiries to Dr J A Franklin on 021-472 1311 extension 3928 or 3361. Application forms from the Medical School on 021-414 4051 quoting reference RA/END/JF/1969. Applications (3 copies) to be returned to the **Senior Assistant Registrar, Medical School, University of Birmingham B15 2TT by 27th April 1989.**

An Equal Opportunities Employer (8917)A

## THE LONDON HOSPITAL MEDICAL COLLEGE (University of London) Postdoctoral Protein Biochemist (plus Honorary Lectureship status for suitable appointee)

Applications are invited for a three year appointment, funded by the Arthritis and Rheumatism Council, to study the oxidative/peptolytic inactivation of alpha-1-antitrypsin in rheumatoid synovial fluid and the potential for anti-inflammatory therapy with recombinant alpha-1-antitrypsins. The project is linked with Professor R W Carrell, Cambridge University.

The position is available now and the salary will be £16,150 – £20,960 inclusive of London Weighting.

Intending applicants should obtain further particulars of the post from Dr Paul Winyard, (Tel: 01-377 7763) or Professor David Blake (Tel: 01-377 7765). CV and names and addresses of two referees should be sent to **Dr P G Winyard, The Inflammation Group, Arthritis and Rheumatism Council Building, London Hospital Medical College, 25-29 Ashfield Street, London E1 1AD within 14 days.** (8895)A

## UNIVERSITY OF OXFORD NUFFIELD DEPARTMENT OF PATHOLOGY POST-DOCTORAL RESEARCH ASSISTANT

A post-doctoral research assistant (1A) is required to investigate the role of respiratory viruses in Sudden Infant Death (Cot Death). The project will involve developing the PCR for detection of specific viral RNA sequences in clinical samples and localisation of these sequences by in situ hybridisation. A knowledge of any of the following would be an advantage, molecular biology, tissue culture, immunohistology and in situ hybridisation.

The post is funded by the Foundation for the study of Infant Deaths for 3 years. Starting salary within the range £9,865-£12,760 dependant on age and experience.

Applications with a full C.V. and the names and addresses of two referees should be sent to: **Dr. K. Fleming, Nuffield Department of Pathology, Level 1, John Radcliffe Hospital, Headington, Oxford: Tel (0865) 817476, from whom further details are available.** Closing date for applications: 27-4-89.

Oxford University is an equal opportunity employer. (8887)A

## PUBLIC HEALTH LABORATORY SERVICE BOARD

PHLS CENTRE FOR APPLIED  
MICROBIOLOGY & RESEARCH  
DIVISION OF BIOTECHNOLOGY  
MICROBIOLOGIST

A graduate technician is required to join the Thermophile Research Section. The successful applicant will be involved in genetic and physiological studies of a wide range of thermophilic microorganisms.

This appointment will be at Basic Grade Microbiologist, with a salary range of £7378-£11073.

NHS terms and conditions

Candidates wishing to informally discuss the post should contact Dr R. J. Sharp, tel: Idmiston (0980) 610391, ext 443.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Tel: (0980) 610391, to whom completed forms should be returned by 21st April 1989 quoting Post No. 0808. (8911)A

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**nature**

**THE INSTITUTE OF CANCER RESEARCH**  
Experimental Unit, Section of Medicine

### Part-time Technician

Technician with experience in histological procedures required to join multidisciplinary team that is using novel immunological techniques to monitor the interaction of tumours with chemotherapeutic drugs and monoclonal antibodies. The work which is based on the use of immunocytochemical techniques will include the use of image analysis for the detection of drug induced damage to the DNA of tumours, and the topoisomerases I and II. In addition the group is interested in the interaction *in vivo* of monoclonal antibodies with growth factor receptors on human tumours that overexpress these receptors.

The post which is part time (30 hours/week) would be suitable for a graduate in a biological science or other suitably qualified person with an interest in the use of histological techniques. While experience in immunocytochemical methodology would be an advantage it is not essential.

Salary will be in the range of £7004 to £9347 for a 30 hour week.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

To apply, please submit a Curriculum Vitae, in duplicate, with names and addresses of two referees to the **Personnel Officer, 17A Onslow Gardens, London SW7 3AL** quoting reference 4.89.T.N.1 (8916)A

**Max-Planck-Institut für molekulare Genetik**  
Innestrasse 73, D-1000 Berlin 33, Germany

### POSTDOCTORAL RESEARCH POSITIONS

are available for scientists in basic research on epidemic bacterial meningitis. Experience in the fields of immunology, protein chemistry or DNA recombinant technology is a prerequisite. Payment is according to BAT and the level of experience. Applications including a full *Curriculum vitae* should be sent to Dr. Mark Achtman at the above address. (W6030)A

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UNIVERSITY OF LONDON

## Sir William Dunn Chair of Experimental Pathology

**Tenable at the United Medical and Dental Schools of Guy's and St Thomas's Hospitals (Guy's Campus)**

The Senate invites applications for the above established Chair which became vacant on the retirement of Professor Colin Adams on 30 September 1988.

The main responsibility of the post is to promote research within the broad field of experimental pathology. Applicants will be considered with an interest in any branch of basic biological science including molecular pathology, cell biology, immunopathology, molecular oncology or neurobiology. The professor may or may not be medically qualified. The successful candidate will be expected to organise, supervise and actively participate in research with members of the Division of Histopathology as well as other Divisions of the United Schools.

The professor will be expected to take an active part in teaching within the pathology courses for undergraduate and postgraduate students especially in the general principles of cellular pathology.

Applications (10 copies) should be submitted to the **Teachers' Section, (N) University of London, Malet Street, London WC1E 7HU**, from whom further particulars should first be obtained.

Prospective candidates wishing to visit the department should contact Professor D A Levison. Tel: 01-407 7600 extension 2591.

Closing date 7 May 1989.

(8896)A

### CANCER RESEARCH CAMPAIGN POSTDOCTORAL SCIENTIST

in the MRC Clinical Oncology and Radiotherapeutics Unit, Cambridge

Applications are invited for a three-year postdoctoral appointment funded by the Cancer Research Campaign and available immediately.

The project involves the study of factors controlling the amplification and expression of genes related to cytotoxic drug resistance in both *in vitro* and *in vivo* tumour model systems. Candidates should have experience in mammalian cell culture and/or molecular biology techniques including *in situ* hybridization.

Enquiries to Dr Peter Twentyman, Tel: (0223) 248011 ext.

Salary £10,460 to £12,760 per annum (under review) on the MRC Non-Clinical Scientific scale.

Applications, including a curriculum vitae and names of 2 professional referees, should be sent quoting reference CRC to:—

**The Personnel Officer**  
MRC Centre  
Hills Road  
Cambridge  
CB2 2QH

**MRC**

to arrive not later than 21 April 1989.

*The Medical Research Council is an Equal Opportunities Employer.* (8894)A

UNIVERSITY OF WARWICK

### LECTURER IN EXPERIMENTAL PHYSICS

Applications are invited for a Lecturer post tenable from 1 October 1989. The person appointed will have research experience in experimental condensed matter Physics. Some preference may be given to persons with experience in the fields of Ceramics and/or Surface Physics. The initial appointment will be on the Lecturer 'A' scale: £10460-£14500 pa (subject to review), with progression to the Grade 'B' scale (to £19310) subject to satisfactory progress.

Application forms and further particulars from the **Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523627)** quoting Ref No 34/A/88/14 (please mark clearly on envelope). Closing date for applications is 5 May 1989.

*An Equal Opportunities Employer*

(8889)A

**INSTITUTE OF PLANT SCIENCE RESEARCH  
CAMBRIDGE LABORATORY  
Molecular Biologist**

The Laboratory is seeking to appoint a Post-doctoral Fellow to carry out research on the genetic engineering of novel pathogen resistances in potato. This work will involve gene isolation and characterization, expression vector construction, and plant transformation. The position would suit someone with a background in genetics, biochemistry or botany, with a particular interest in the application of genetic engineering to crop plants. Full training will be given

The post is funded for two years. Initially the successful applicant will be based at the Cambridge Laboratory but in March 1990 will transfer to new laboratories at the John Innes Institute, Norwich.

The salary will be on the HSO scale £10,026 – £13,400, with a non-contributory superannuation scheme.

Applications with curriculum vitae and names and addresses of 3 referees should be sent to **Mrs L Cliff, Personal Officer, IPSR Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB**, quoting reference **MG/357**, by **21st April 1989**.

*The Institute is an Equal Opportunities Employer* (8915)A

**UNIVERSITY OF GLASGOW**

**Department of Electronics and Electrical Engineering  
Lecturer in Bioelectronics**

This lectureship provides a remarkable opportunity to join a team of electronic fabrication engineers, cell biologists and biochemists who are attempting to explore the interface between biological moities and electronic circuits with the ultimate ambition of progress in embryology, understanding neural networks and development of prosthetic devices. The Group enjoys substantial support from BP Venture Reserch Unit and SERC.

Honours degree lecture courses in Bioelectronics are being developed in conjunction with University Departments of Life Sciences.

Candidates should have an honours degree in electronics, electrical engineering or physics: postgraduate experience in bioelectronics would be helpful though not essential. This appointment arises through expansion of the Department.

Salary will be on the scale for Lecturers within the range £9260 – £19310 p.a. (under review). Placement will be according to experience and qualifications.

Further particulars may be obtained from the **Academic Personnel Officer, University of Glasgow, Glasgow G12 8QQ**, with whom applications (8 copies) should be lodged by **24 April 1989** giving the names and addresses of three referees. Applicants wishing to visit the Department would be welcome to do so by arrangement with Professor John Lamb.

In reply please quote Ref 6300/1

(8936)A



**McMaster University  
CHAIRMAN,  
DEPARTMENT OF BIOLOGY**

Applications are sought for the position of Chairman, Department of Biology. The candidate should have an outstanding research record in developmental biology and possess excellent leadership qualities.

The appointment is available July 1, 1989, and will be at the rank of Associate Professor or Professor, salary and rank to be commensurate with qualifications and experience. Applications should include a detailed curriculum vitae and the names of three referees. Applications or requests for further information should be addressed to:

**Dr R.F. Childs, Dean of the Faculty of Science  
McMaster University, Hamilton, Ontario, L8S 4K1**

\* In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada. McMaster is an equal opportunity employer. (NW3562)A

**POSTDOCTORAL  
RESEARCH FELLOW**

**UEA  
NORWICH**

Applications are invited for the above post in the School of Biological Sciences, commencing 1st July 1989 or as soon as possible thereafter for a fixed term of 36 months, to study the nature and significance of electrical phenomena associated with the systemic induction, by wounding, of proteinase inhibitors in tomato plants. Applicants should have a PhD or equivalent experience, preferably in electrophysiology, but other relevant subject areas will be considered. Previous experience of work with plants is not essential. Salary will be on the RA1A scale (£9,845-£11,680) according to qualifications and relevant experience. The project is funded by a grant from the Agricultural and Food Research Council to Drs. J. F. Thain and D. C. Wildon, to whom enquiries should be addressed (Tel: (0603) 56161 ext. 2257).

Applications, giving full curriculum vitae and the names of two referees, should be sent to **Drs. J. F. Thain and D. C. Wildon, School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ**. Closing date for applications 5th May 1989. (8929)A

**DEPARTMENT OF SURGERY,  
UNIVERSITY OF EDINBURGH  
and SOUTH-EAST REGIONAL  
CENTRE OF THE SCOTTISH  
NATIONAL BLOOD  
TRANSFUSION SERVICE  
RESEARCH FELLOW  
and PART-TIME  
RESEARCH ASSISTANT**

Applications are invited for the above posts which are funded by a grant awarded to Dr. K. James, Dr. C. V. Prowse and Dr. D. B. L. McClelland under the MRC AIDS Directed Programme. The aim of this project is to develop human monoclonals to HIV for use both in AIDS research and patient management.

Preference will be given to applicants with hybridoma, virological and immunoassay skills.

The posts are funded for three years with starting salaries according to age and experience as follows:—

Research Fellow (Scale 1A) up to £11,680

Research Assistant (Scale 1B) half time — £5,230

For further details of these posts contact either Dr. K. James (667 1011, ext. 2287) or Dr. C. V. Prowse (229 2585 ext. 298). Formal applications in writing with a full C.V. and the names and addresses of two referees should be sent to **Dr. Keith James, Department of Surgery, University Medical School, Teviot Place, Edinburgh EH8 9AG**. Closing date — April 30 1989.

Please quote reference no. 5656 (8885)A

**MEDICAL RESEARCH  
COUNCIL**

**DUNN NUTRITION UNIT  
CAMBRIDGE**

**TENURED SCIENTIFIC  
STAFF POST —  
MICROBIOLOGY**

Applications are invited for a permanent non-clinical scientific staff post with the M.R.C. An experienced microbiologist is required to lead a team specialising in the biochemistry and physiology of the anaerobic flora of the hind gut working at the Dunn Clinical Nutrition Centre.

Salary will be on the scales for university non-clinical academic staff. Please send a c.v. quoting ref DCNC/TS, together with the names of at least two referees to Steve Allen, MRC Dunn Nutrition Unit, Downham's Lane, Milton Rd., Cambridge, CB4 1XJ. Closing date for application Friday 21 April 1989.

The MRC is an Equal Opportunity Employer. (8892)A

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## STAFF SCIENTISTS PROTEIN DESIGN LABS

Protein Design Labs is a biotechnology company based on the concept of protein engineering. We are applying recent developments in molecular biology, immunology, protein chemistry and computer modeling to create novel proteins for the treatment of human disease, especially autoimmune disease and cancer. Our first product, a humanized antibody for the prevention of organ transplant rejection, will enter clinical trials soon. We have an outstanding group of scientists and scientific advisors, and an excellent location near Stanford University.

Additional staff scientists and research assistants are needed in all areas. We offer competitive compensation, stock in our growing company, and an opportunity to do exciting science.

**Send CV to Dr. Cary Queen, Protein Design Labs, 3181 Porter Drive, Palo Alto, CA 94304. EOE.**

(NW3579)A

## DEPARTMENT OF VETERANS AFFAIRS

### VETERANS HEALTH SERVICES AND RESEARCH ADMINISTRATION

## DIRECTOR MEDICAL RESEARCH SERVICE

The Department of Veterans Affairs invites applications for the position of Director, Medical Research Service. The Medical Research Service currently has a budget of about \$200 million and supports biomedical and behavioural sciences research of over 2500 VA scientists, 70 percent of whom are physicians. It is strongly committed to support of patient-oriented research across the entire spectrum from basic science to clinical applications of new knowledge. The Director is responsible for policy development, peer review management of all investigator-initiated research studies and budget formulation and distribution. Candidates are expected to be nationally recognized scientists and they must have an established record of scholarly achievement. The position is only open to U.S. citizens who hold an M.D. degree. Salary is \$75,500 with additional special pay up to \$24,000.

Qualified applicants are invited to send a curriculum vitae, bibliography and the names, addresses and telephone numbers of three respondents by 15 May 1989 to: **Richard J. Greene, M.D., Ph.D., Assistant Chief Medical Director for Research and Development (15), Department of Veterans Affairs, 810 Vermont Avenue, N.W., Washington, D.C. 20420.**



**Veterans  
Administration**

(NW3541)A

### POSTDOCTORAL FELLOW/ INSTRUCTOR/ASSISTANT PROFESSOR Requirements: Ph.D. degree in molecular and/or cell biology

The Department of Medicine at Brigham and Women's Hospital and Harvard Medical School, Boston, MA is seeking applicants with a Ph.D. degree in molecular and/or cell biology for a position in the Molecular and Cellular Vascular Research Laboratory. The laboratory employs a multidisciplinary approach with emphasis on qualifications. Recognized excellence in basic investigation is deemed essential. Position available: July 1, 1989. Annual Salary: to be negotiated. Send curriculum vitae and references to:

**Dr. Victor J. Dzau, M.D., Chief, Molecular and Cellular Vascular Research Laboratory, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115. Telephone: (617) 732-6628; FAX: (617) 732-6961.**

(NW3553)A

### FULL-TIME POSTDOCTORAL POSITION OR ASSISTANT RESEARCH PHYSIOLOGIST POSITION

Available immediately for two years (with possibility for renewal), for a biophysicist trained in cellular electrophysiology to study calcium action in neurons. Annual salary \$23,196 - \$28,920 for postdoc, \$36,600 - \$45,700 for Assistant Research, depending on experience. Photosensitive calcium chelators will be used to probe the role of calcium in transmitter release and plasticity at central and peripheral synapses, and in activating ionic currents. Experimental preparations include neuromuscular junction, ganglionic and isolated neurons, and the squid giant synapse using a local squid holding facility. A new calcium imaging system on an upright microscope is available to trace changes in presynaptic calcium during electrical activity, and explore ionic mechanisms of facilitation and potentiation.

Before 11 May 1989, send resume, reprints, and two names of references to: **Dr. Robert Zucker in the Neurobiology Division of the newly designated Molecular and Cellular Biology Department, University of California, Berkeley, CA 94720.**

*An Equal Opportunity Affirmative Action Employer*

(NW3545)A

## POSTDOCTORAL POSITIONS

The CNRS has created

a

### PLANT SCIENCE INSTITUTE (INSTITUT DES SCIENCES VEGETALES)

on its Gif Research Centre (the main CNRS Campus for biological research in the Paris area). Excellent facilities for molecular biology and plant work (growth cabinets, greenhouses, etc.) will be provided. From late Spring 1989 the ISV will start operation in the completely renovated laboratories. The aim of the ISV is to conduct research in the field of molecular and cellular biology of plant growth, differentiation and development, taking into consideration also potential applications. Several of the already selected programmes will focus on the recognition and transduction of signals leading to these processes in microbe-plant interactions. Adam Kondorosi has been named director and the establishment of research groups, headed among others by Jacques Tempé, Bruno Gronenborn and Eva Kondorosi, is underway.

Two postdoctoral positions for plant molecular biology research in the field of molecular biology of microbe-plant interactions are available. Applications should be sent to: **the Director of the Institut des Sciences Vegetales, CNRS, Avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex, France. (W6037)A**

### University of Manchester, Department of Chemistry Postdoctoral Research Associate in Molecular Modelling

Applications are invited for a three year Postdoctoral Research Associate Post funded by Shell Research Limited. The successful applicant will study aspects of molecular similarity and molecular recognition on theoretically determined reaction pathways. Experience in the development and use of quantum mechanical codes is desirable for this position. The initial salary will be up to £14,000 p.a. **Further details and application form may be obtained from Professor I.H. Hillier, Department of Chemistry, University of Manchester, Manchester M13 9PL.**

(8937)A

## FACULTY POSITION — X-RAY CRYSTALLOGRAPHY

The Department of Biochemistry, Medical School at the University of Minnesota invites applications for a tenure-track faculty position at the Assistant or Associate Professor level. Candidates with professional distinction in research and writing and demonstrated effectiveness in teaching and advising will be considered for an Associate Professor position. Candidates demonstrating involvement in quality research accepted or published in peer-reviewed journals will be considered for an Assistant Professor position. Applicants should have a Ph.D. degree with expertise in x-ray crystallography and structural analysis of macromolecules. The successful applicant will be part of a group of scientists working in structural biology associated with the William F. Dietrich Chair program. The Structural Biology group complements a core of biophysical scientists in several departments in the Medical School and in the College of Biological Sciences. These departments maintain strong graduate programs in biophysics and molecular biology. The new opening includes ready access to state-of-the-art x-ray diffraction facilities and the Minnesota Supercomputer Institute. Applications must be postmarked no later than June 1, 1989. Send curriculum vitae, a concise plan for future research, and the names of three references to:

**Leonard J. Banaszak, Center for Structural Biology  
Department of Biochemistry, Medical School  
4-225 Millard Hall, 435 Delaware St. S.E.  
University of Minnesota  
Minneapolis, MN 55455**

The University of Minnesota is an equal opportunity educational employer, and specifically invites and encourages applications from women and minorities.

(NW3567)A

### POSTDOCTORAL RESEARCH ASSOCIATE

position available summer/fall 1989 in time-resolved fluorescence spectroscopic studies of cell membranes and lipid bilayers with focus on drug-membrane and protein-lipid interactions.

Applicants should be recently qualified Ph.D.'s with a general background in either **physical chemistry** or **biophysics** with interest or experience in fluorescence spectroscopy, lipid bilayers and cell membranes. The department has a large, well equipped membrane research group with interests in membrane physical properties and related fields.

Send CV and the names of three references to: **Dr. C. D. Stubbs, Department of Pathology and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107.**

Equal Opportunity Employer  
(NW3573)A

### MURDOCH UNIVERSITY Perth, Western Australia POSTDOCTORAL RESEARCH FELLOW — THERMODYNAMIC MODELLING SCHOOL OF MATHEMATICS AND PHYSICAL SCIENCES

Applications are invited for the position of Postdoctoral Research Fellow to assist with a research project in Thermodynamic Modelling. The work involves the theoretical and practical development of a computer model for the control of a large scale metal production plant in Kalgoorlie, Western Australia. The project will involve work mainly on campus in Perth but also on-site in Kalgoorlie. Accommodation in Kalgoorlie will be provided free of charge.

A graduate in chemistry is preferred but graduates in chemical engineering or metallurgy will be considered. Fortran Programming ability and a knowledge of chemical thermodynamics are required.

The position is available for two years. (Ref 1134)

Further information may be obtained from Dr Peter May (tel (09) 332 2203, fax (09) 332 2507).

Salary: A\$26,617 to A\$30,360 per annum.

Applications in duplicate, quoting reference number, including full personal particulars, details of tertiary qualifications and experience, a curriculum vitae and academic transcript and the names and addresses of two professional referees should reach the Chief Personnel Officer, Murdoch University, Murdoch, Western Australia, 6150 by Friday 19 May 1989.

Applicants resident in the United Kingdom, Europe or Africa, at the time of application should also forward One further copy to Appointments (36228), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK.

Murdoch University is an Equal Opportunity Employer.  
(W6032)A

### FACULTY OF MEDICINE AND HEALTH SCIENCES

UNITED ARAB EMIRATES  
UNIVERSITY

### ASSISTANT/ASSOCIATE PROFESSOR/PROFESSOR\* DEPARTMENT OF MEDICAL ANATOMY

The Faculty of Medicine and Health Sciences is now in its third year of operation having accepted its first class of medical students in September 1988 following two years of premedical study. The Faculty of Medicine utilizes two affiliated hospitals in Al Ain Tawam Hospital and Al Ain Hospital, with a total of 1050 beds. Currently housed in temporary facilities, it is in the process of finalizing plans for the construction of a teaching and research facility of international stature to be completed in 1991. The language of instruction is English.

Candidates applying for these positions must have demonstrated interest in teaching and research. Emphasis is placed upon candidates with an interest and experience in a problem solving approach to medical education. In addition, candidates must possess a medical degree and/or PhD degree as appropriate to post or an equivalent higher professional degree from an accredited university or institution of higher learning. Preference will be given to candidates possessing a higher qualification.

Salary and liberal benefits are offered, commensurate with qualifications and level of responsibility. If you are seeking professional growth and an excellent work environment, send detailed resume, copies of certificates and passport and the names of three referees familiar with recent work history to: The Dean, Faculty of Medicine and Health Sciences, UAE University, PO Box 15551, Al Ain, United Arab Emirates.

\*The rank of Professor does not carry Chairmanship with it. Applications should be received within one month following publication date. All positions will remain open until filled. W(6029)A

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### SENIOR SCIENTISTS

Innovative, well funded medical diagnostic device start up co. seeks founding scientists:

- Polymer/Organic Chemist with Ph.D. and 10+ yrs industry exp. in medical R&D. Broad knowledge of physical chemistry and biology req.
  - Assay Develop. Scientist with Ph.D. in protein chemistry or equiv. and 8+ yrs industry exp in immunoassay and diagnostic product develop. Broad knowledge of organic and protein chemistry req.
- Resumes to: **R&D Dpt., Biocircuits Corp., 1450 Rollins Rd., Burlingame, CA 94010. EOE/AA.**  
(NW3578)A

### PROTEIN BIOCHEMIST

Perform purification and characterization of novel proteins. Proficiency with analytical methods for chemical, immunological, and structural determinations required. Expertise in separation techniques desirable.

**Protein Polymer Technologies, Inc.** Attn: Dr. Cappello, 10655 Sorrento Vly. Rd. San Diego, CA 92121

(NW3580)A

**Agricultural Research Service (ARS)  
U.S. Department of Agriculture (USDA)  
Chemical/Biochemical Engineers  
Northern Regional Research Center  
Peoria, Illinois**

ARS is seeking two postdoctoral research associates with no more than 3 years' postdoctoral experience to join a research group studying the conversion of agricultural commodities into biocontrol products. Both positions are open immediately. One associate will conduct research on luminescence-based sensors for in-line monitoring and control of fermentation processes. Collaboration with researchers in the chemistry and electrical engineering departments at the University of Illinois will be necessary. Applicants must have excellent communication skills, as well as knowledge of data acquisition and control strategies. Experience with microbial processes and analytical (immunology) techniques is desirable. The other associate will develop technology required to mass produce and formulate products to biologically control plant diseases and weeds. Experience in studies of transport processes, interfacial phenomena, and microbial ecology are desirable. Salary is commensurate with experience \$32,669-45,542. Send curriculum vitae marked **9N012** with names and telephone numbers of three references to **Dr. P. J. Slininger, NRRC-ARS-USDA, 1815 N. University Street, Peoria, IL 61604**, by June 1, 1989. ARS is an equal opportunity employer. (NW3576)A

**UNIVERSITY OF  
essex**

**Department of Biology  
SENIOR RESEARCH OFFICER**

Applications are invited from postdoctoral candidates for a post of Senior Research Officer to take part in a project funded by the SERC for three years to develop immunological methods for large scale fractionation of cells. Experience of monoclonal antibody production is required. The starting salary will be up to £11,680 per annum on Grade 1A. Further support may be obtained from industrial collaborations.

Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (R/864/N), University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, from whom further particulars may be obtained, by **27th April 1989**. (8928)A

**SCIENCE IN EUROPE  
27th April 1989**

**nature**  
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

This special feature issue of *Nature* will focus on the present pattern of science in Europe.

Vacancies, fellowships, symposia, conferences, lectures, workshops, announcements will all benefit from the extra interest generated by this truly "European" issue.

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Tel: (089) 26 50 32  
Fax: (089) 26 93 24

Paris—Clare Newell:  
Tel: (1) 40 53 03 39

**UMIST**

**Department of Biochemistry and Applied Molecular Biology  
Lignocellulose Degradation by Actinomycetes**

**RESEARCH ASSOCIATE**

A postdoctoral position is available for three years to participate in a programme to investigate novel extracellular enzymes involved in the degradation of lignocellulose by actinomycetes. The opportunity exists to combine an interesting research programme with wider commercial applications. Applications from candidates with a particular interest in lignocellulose structure and degradation products would be welcomed. Experience of analytical techniques such as HPLC would be an advantage. Commencing salary will be within the scale: £9,865-£15,720 per annum. Letters of application, including full curriculum vitae and the names of two referees, should be sent to: **Professor P M A Broda, Department of Biochemistry and Applied Molecular Biology, UMIST, P O Box 88, Manchester M60 1QD**, to whom informal enquiries may be made on 061 236 3311 ext 2119. Please quote reference BIO/R/34. (8935)A

**Harvard Medical School  
Genetics Division at The Children's Hospital  
A Postdoctoral Position**

is available in Human Genetics. The candidate should have experience in molecular techniques. He/she will work on structural/functional aspects of the human sex chromosomes. The position is funded for at least two years. Please send CV and three letters of recommendation to: **Dr. Ulrich Muller, Division of Genetics, The Children's Hospital, 300 Longwood Avenue, Boston, MA 02115**.

Harvard Medical School is an Equal Opportunity/Affirmative Action employer. (NW3577)A

**Antisense RNA  
Regulation**

Postdoctoral position (Sept. 1, 89) in yeast molecular biology on use of antisense RNA for gene regulation. Experience in recombinant DNA required. Send C.V. and names of 3 references to: **Dr. G. H. Rank, Dept. of Biology, Univ. of Sask., Saskatoon, Sask., Canada, S7N 0W0**. (NW3551)A

**POSTDOCTORAL POSITION**

We are seeking an experienced membrane biochemist who will isolate the complete bacterial flagellar motor and determine its structure by electron microscopy. The Structural Biology Laboratory at Brandeis has extensive facilities for electron microscopy and image analysis, and will provide as part of this project training in these techniques. Inquiries and applications, including a CV and names of three references, should be sent to:

**Dr. David DeRosier, Rosensteel Basic Medical Sciences Research Centre Brandeis University,  
P.O. Box 9110, Waltham, MA 02254-9110**

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(NW3560)A



UNIVERSITY OF ST ANDREWS  
DEPARTMENT OF BIOCHEMISTRY  
AND MICROBIOLOGY

### POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a 3-year SERC funded postdoctoral position to work on the molecular genetics of nitrite reduction in barley. Applicants should preferably have a background in molecular biology or genetics.

Starting salary within range £9,865 to £11,680 per annum, on the 1A scale.

Informal enquiries, and formal applications with a *Curriculum Vitae* and the names of two referees, should be sent to **Dr J L Wray, Plant Molecular Genetics Unit, University of St Andrews, Sir Harold Mitchell Building, St Andrews, Fife KY16 9TH**, (Tel 0334 76161 Ext. 7253). (8907)A

### POSTDOCTORAL POSITION, NEUROPHYSIOLOGIST.

Position available immediately to work on the neuronal basis of learning in invertebrate animals. Candidate should be experienced in extracellular and intracellular recording and will work in association with a group studying behavioral, pharmacologic, and immunocytochemical aspects of conditioning in crustacea (*J. Neurosci.* (88) B, 2907-2912). We offer an excellent benefits package and a salary of \$24,000 per year.

Please send C.V. and the names of two references to:  
**Prof. Richard D. Feinman, Department of Biochemistry, SUNY Health Science Center at Brooklyn, 450 Clarkson Avenue, Box 8, Brooklyn, NY 11203.**

EO/AA Employer DMC CR0255 (NW3575)A

## Hoping to change jobs?

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## AWARDS

# Sandoz Prize for Immunology

The prize will be worth US \$ 100 000 (US \$ 20 000 personal recognition/US \$ 80 000 support for research programme), and will be sponsored by SANDOZ LTD., Basle, Switzerland with the purpose of encouraging research in all areas of immunology with special emphasis on clinical immunology, including autoimmune diseases, cancer immunology, immunity to infectious diseases, transplantation immunology and discoveries in immunology leading to therapeutical applications.

Members of the Jury are G. Ada, J.-F. Bach, T. Honjo, P. Marrak, H.O. McDevitt, J.J. van Rood, R. Zinkernagel, and two representatives of Sandoz Ltd. The prize will be awarded on the occasion of an important Immunology Meeting in 1990.

Applications in English should comprise a summary of the research work of 3-5 pages, curriculum vitae, bibliography, experimental original papers separate from reviews and chapters, and reprints of not more than 3 key published papers in English or with extended summaries in English.

Individuals and research teams are invited to submit their applications not later than 30th June, 1989 to Sandoz Prize for Immunology, P.O. Box 182, 4013 Basel, Switzerland.



## FELLOWSHIPS



## EUROPEAN SOUTHERN OBSERVATORY

Organisation Européenne pour des Recherches Astronomiques dans l'Hémisphère Austral Europäische Organisation für astronomische Forschung in der südlichen Hemisphäre

## FELLOWSHIP AT LA SILLA

A position is available at La Silla for a post-doctoral fellow with an interest in observational astronomy. Experience with IR instrumentation or optical photometry will be an advantage.

The successful applicant will be expected to spend not more than 50% of his/her time in support related activities and the rest of the time doing scientific research. The presence at La Silla will be for at least 150 nights per year. ESO fellowships are granted for a period of one year normally renewed for a second period and exceptionally renewed for a third and final year.

The facilities on La Silla consist of 15 telescopes including the SEST 15-m submillimeter antenna, and the new 3.5m NTT. The computing facilities comprise an HP1000 system with full image processing capabilities (IHAP), a VAX 11/750 mainframe, and three SUN 4/110 workstations for image processing (MIDAS).

Close to 20 astronomers, including staff members, fellows and students, work at La Silla. The research projects currently pursued by the astronomical staff at La Silla include low mass star formation (Herbig-Haro objects, molecular outflows, T Tauri stars), OH/IR stars, symbiotic stars and proto-planetary nebulae, coronal activity in late type stars, chemistry of molecular clouds, formation of massive stars and starburst activity, dynamics of elliptical galaxies, active nuclei, QSOs and gravitational lensing, and observational cosmology.

Applicants normally should have a doctorate awarded in recent years. The monthly basic salary will be not less than DM 4190 to which are added 7% for pension purposes and a non resident allowance of 30-45% as well as a mountain allowance of 5-10%. Applications should be submitted to ESO not later than May 15, 1989. Applicants will be notified in July 1989. The ESO Fellowship Application Form should be used and be accompanied by a list of publications. In addition, three letters of recommendation should be obtained from persons familiar with the scientific work of the applicant. These letters should reach ESO not later than May 15, 1989.

Enquiries, requests for application forms and applications should be addressed to: **European Southern Observatory, Fellowship Programme, Karl-Schwarzschild-Straße 2, D-8046 GARCHING b. München, Federal Republic of Germany** W(6026)E

## POSTDOCTORAL FELLOWSHIP

NIH Staff Fellow/Senior Staff Fellow position is available at the National Institute on Alcohol Abuse and Alcoholism, for studies of neurotransmitter and hormone receptors and their interactions with cytokines and growth factors. Preference is given to applicants with experience in molecular biology, protein purification and receptor biochemistry/pharmacology. Salary is commensurate with experience. Only U.S. citizens or permanent residents are eligible for this position.

Send CV and name of three references to:

**Dr. George Kunos**  
Laboratory of Physiologic and  
Pharmacologic Studies NIAAA  
12501 Washington Avenue  
Rockville, MD 20852  
Phone: (301) 443-1234



NIAAA IS AN EQUAL OPPORTUNITY EMPLOYER

(NW3561)E

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
National Institute of Mental Health

## Extramural Research Staff Fellowship Program

The National Institute of Mental Health (NIMH) in the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA), Public Health Service announces a unique opportunity for both developing and well-established scientists to work closely with senior scientists and administrators in guiding its extramural research support programs. The Extramural Staff Fellowship Program will provide a limited number of fellowship appointments in the biomedical, neuroscience, behavioral, clinical and services research areas. Extramural staff fellowships are intended to give talented scientists at an early, mid-point, or senior level in their career an opportunity to participate in and bring new ideas to the scientific research supported at NIMH. Opportunities may also be available for extramural staff fellows to participate in the relevant research projects in collaboration with the Institute's intramural research program or with outside organizations. Fellows must hold an M.D., Ph.D., or equivalent degree. Salary will be in the \$29,000 to \$75,500 range, depending upon training, post-doctoral research experience, and the scope and complexity of the research skills required. Fellows would also be eligible for full Government benefits and leave. U.S. citizenship or permanent residency status is required. Appointments will be made for an initial period of up to two years, and may be extended. Positions will be located in Rockville, Maryland. Interested persons should contact either NIMH Director (Dr. Lewis Judd) or the Deputy Director (Dr. Alan Leshner) at 301-443-3673, or may submit a curriculum vitae in confidence to 5600 Fishers Lane, Room 17-95, Parklawn Building, Rockville, Maryland 20857.

ADAMHA is an Equal Opportunity Employer.

(NW3538)E

## UNIVERSITY OF OXFORD



## NUFFIELD MEDICAL RESEARCH FELLOWSHIP

A Nuffield Medical Research Fellowship, tenable for a fixed period of two years in the first instance with the possibility of renewal for a further and final fixed period of one year, will be available from 1 October 1989. The successful candidate (who will not normally be expected to have more than five years of experience after completing a medical qualification or a doctorate) will be required to carry out a research project in a clinical academic or medical sciences department of the University. Salary (under review) on the scale £13,470-£15,510 if medically qualified, or £9,865-£12,150 if not. The appointment will be held in conjunction with a Junior Research Fellowship at New College. Further particulars may be obtained from the

**Senior Assistant Registrar,  
The Medical School Offices,  
John Radcliffe Hospital,  
Headington, Oxford, OX3 9DU**

to whom applications (including details of the research project agreed with the head of department in which it would be carried out) must be sent by 5 May 1989. (8919)E

*The University is an Equal Opportunity Employer*

## EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION  
SHORT TERM FELLOWSHIPS  
in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from **Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G.** (W5706)E

## INTERNATIONAL CANCER RESEARCH FELLOWSHIP

The Hayashibara Mutual Aid Fund, a non-profit making organization within the Hayashibara Group announces a fellowship program. Two awards will be made annually. Fellowship will be taken up at the newly opened Fujisaki Cell Center which is devoted for the basic and applied research related to the problems in human cancer. The fellowship is normally made for one year and is renewable for up to five years. The fellowship will be expected to commence within six months of the announcement of the awards. The awards are for high quality research work in one of the three categories described below.

1. Fundamental Leukemia-Lymphoma Research,
2. Cytokine-Lymphokine Research,
3. Hematopoietic Cell Cultures.

Qualified person who has a Ph.D., an M.D. or equivalent qualifications will be awarded on merit to suitably qualified research proposal and experiences. Additional information and application forms will be obtained from: **Jun Minowada, M.D., Director, Fujisaki Cell Center, 675-1, Fujisaki, Okayama 702, Japan.** (W5829)E

THE UNIVERSITY OF  
THE SOUTH PACIFIC

Alafua Campus, Western Samoa

FELLOWSHIPS IN THE  
INSTITUTE FOR  
RESEARCH, EXTENSION  
AND TRAINING IN  
AGRICULTURE (IRETA)

The following Fellowships are tenurable at the Institute (IRETA) which is centred with the School of Agriculture on the University's Alafua Campus in Apia, Western Samoa, and serves eleven Pacific Island nations in the Region. Citizens of EEC and ACP countries only are eligible to apply.

The vacancies are advertised pending final confirmation of funding which has been approved in principle but not finally confirmed yet.

Fellow in Plant Tissue Culture  
(Post 89/15)

A unit in Plant Tissue Culture has been set up within IRETA with the objective of providing a service to the Governments of the countries of the Region to facilitate the safe introduction, multiplication and dissemination of improved cultivars of commercial crops using tissue culture and to provide a germplasm store for some of the crops important to the region. The unit is working with Sweet Potato, Ipomea; Colocasia, taro; Banana, Vanilla and Coconut embryo tissue, and has all the basic facilities for this work. It is planned to expand the work and funds are available for the modification and equipping of culture and storage rooms and for a post quarantine screen house. The proposed plan of work includes the development of tissue culture procedures for other crops and the introduction where possible of local indexing of virus free materials together with training programmes and workshops for agriculture staff of the Region and students in the School of Agriculture.

Recruitment of a senior experienced Fellow who will be responsible to the Director of IRETA for the work of the unit is in progress. The successful applicant for this post will be responsible to the Senior Fellow and assist her or him with the programme. Applicants should possess a good first degree in Botany, Horticulture or Agriculture and some experience of tissue culture or a higher degree taken in the field of tissue culture.

## Fellow in Applied Statistics (Post 89/16)

The appointee will be responsible to the Director of IRETA for the provision of a statistical design and analysis service to the Agricultural Departments of the South Pacific Region and to undertake formal

and non formal teaching in experimental design and analysis and the use of microprocessors in the field of agricultural experimentation and survey. The Alafua campus has standardized on IBM computers and has IBM, AT and XT models for this work together with a network of six stations for teaching purposes and funds to extend this basic configuration. The University has at its Laucala Campus a Vax computer accessible by post and shortly by radio satellite data transfer.

Applicants should possess a good first degree in Agriculture, Science or Mathematics and a higher degree in Agricultural Experimentation and Statistics or Computing. Experience in the fields of experimental design and statistical analysis and in the use of microprocessors and the software appropriate to IBM machines is essential.

Salaries for the above positions will be in accordance with qualifications and experience in the following scales: Junior Lecturer WS\$24,353-WS\$27,324; Lecturer II WS\$28,414-WS\$31,793; Lecturer I WS\$32,917-WS\$44,735; Senior Lecturer WS\$46,166-WS\$53,946. The University also provides gratuity amounting to 15% of basic salary; appointment allowance; partly furnished accommodation at a rental of 12.5% of salary; and a contribution of 10% of basic salary towards an approved superannuation scheme. The University has also introduced a home currency option to members of staff who are based outside the home countries to assist them with exchange rate fluctuation. Appointment will be for a contract period of three years with a possibility of extension for a further two years.

Further information may be obtained from the Assistant Registrar (Staffing) at the University Laucala Campus, Suva, Fiji (Telephone 313900; Telex FJ2276; 1 (679) 301305).

Candidates should send THREE COPIES of their curriculum vitae with full personal particulars names and addresses of three referees and date of availability. In order to expedite the appointment procedure applicants are advised to ask their referees to send confidential reports direct to the University without waiting to be contacted. Applications should be sent to the Secretary, School of Agriculture, University of the South Pacific, Alafua Campus Private Mail Bag, Apia, Western Samoa to reach him no later than 28 April 1989. Applicants residing in the UK should also send a copy to the Appointments Office, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W6033)E



# POST-DOCTORAL FELLOWSHIPS

The Imperial Cancer Research Fund is one of the largest independent cancer research institutes in Europe employing approximately 400 scientists and clinicians. It has a wide-ranging programme in fundamental, applied and clinical cancer research.

There are approximately seventy research groups encompassing the major disciplines of cellular and molecular biology including biochemistry, genetics, immunology, and virology. Most are housed at the main building in Central London; there are also research units at Clare Hall, North London and at Dominion House attached to St Bartholomew's Hospital. ICRF's external clinical units based in major teaching hospitals form the link between fundamental research and clinical activities.

Each research group works independently. All have access to centralized services including cell production, oligonucleotide and peptide synthesis, amino acid and DNA sequencing, a library of DNA probes and somatic cell hybrids for gene mapping, experimental animal breeding and maintenance including transgenic mice, electron microscopy, and a large computer facility.

## Fellowships

Approximately 40 post-doctoral fellowships are available each year to scientists of any nationality. Most of these are three-year fellowships, but visiting fellowships for a period of one or two years are also offered.

Salaries for post-doctoral fellows are in the range of £12,500-£16,000, dependent on age and experience. This is subject to UK tax but avoidance of double taxation agreements exist with all countries of the EEC, the Commonwealth and the USA. Salaries for Visiting Fellows are dependent on age and experience. Visitors are entitled to medical and dental care under the National Health Service. ICRF will provide assistance with relocation expenses.

The projects currently available are listed below.

Applications giving the relevant project number and including a full Curriculum Vitae should be sent by 12 May 1989 to:  
**Recruitment Officer (Ref: 108/R), Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX.**

1. **John Armstrong**  
Role of ras-like G-proteins in membrane traffic of *S. pombe*.
2. **Lionel Crawford (Cambridge)**  
Transformation of human keratinocytes by human papillomaviruses and oncogenes.
3. **Clive Dickson**  
The function of *int-2* and related growth factors in tumourigenesis and normal fetal development.
4. **Agamemnon Epenetos**  
Design of chimeric anti-tumour immunoconjugates.
5. **Michael Fried**  
Mammalian gene organization and expression.
6. **Michael Fried**  
Molecular analysis and cloning of amplified DNA.
7. **Stephen Goodbourn**  
Regulation of human  $\beta$  interferon expression.
8. **Bridget Hill**  
Drug resistance mechanisms in cells pre-exposed to X-irradiation and expressing atypical multidrug resistance.
9. **Bridget Hill**  
Modified DNA repair capacity and cisplatin resistance.
10. **Nancy Hogg**  
Analysis of monocyte molecules including adhesion receptors in immune responses.
11. **Michael Horton**  
Molecular analysis of bone cell specific genes.
12. **Nicholas Jones**  
Transcriptional regulation by the adenovirus E1A gene product.
13. **Peter Karran**  
DNA mismatch repair in human cells.
14. **Roger King**  
Growth factors and breast cancer cell proliferation.
15. **Roger King**  
Use of transfected oestradiol receptor (ER) cDNA to study mechanisms of oestrogen action.
16. **Kevin Lee**  
Regulation of transcription by cyclic-AMP.
17. **Mark Meuth**  
Fine structure deletion mapping of a potential origin of DNA replication in the hamster *APRT* locus.
18. **Malcolm Parker**  
Molecular aspects of steroid hormone action.
19. **Gordon Peters**  
Functions of the *int-2* and *hst* oncogenes in tumourigenesis and embryogenesis.
20. **Denise Sheer**  
Molecular genetic analysis of selected solid tumours in children.
21. **George Stark**  
Regulation of gene expression by interferon.
22. **George Stark**  
Molecular analysis of mammalian gene amplification.
23. **Michael Sternberg**  
Computer algorithms to detect protein sequence homology.
24. **Joyce Taylor**  
Use of synthetic peptides and glycopeptides as immunogens in cancer.
25. **Joyce Taylor**  
Oncogenic transformation of human mammary epithelial cells: an *in vitro* model for studying breast cancer.
26. **Graham Warren**  
Re-assembly of the Golgi apparatus in a cell-free system.
27. **Roger Watson**  
*C-myc* expression in haemopoietic cells.
28. **Stephen West**  
Homologous pairing and formation of synaptic intermediates between regions of duplex DNA by RecA protein.
29. **Stephen West**  
Characterization of an endonuclease from *S. cerevisiae* that resolves Holliday junctions in DNA.
30. **Roland Wolf (Edinburgh)**  
Identification of human genes involved in drug resistance towards alkylating agents by complementation in yeast.
31. **Richard Wood**  
The biochemistry of DNA excision repair in human cells.
32. **Nicholas Wright/Susan Kirkland**  
Control of differentiation in colorectal carcinoma cells.
33. **Bryan Young**  
Molecular analysis of Philadelphia chromosome positive leukaemias.

I M P E R I A L  
CANCER RESEARCH FUND

(8938)E

## FELLOWSHIPS continued

# UNIVERSITY of GUELPH

## DEPARTMENT OF BIOMEDICAL SCIENCES POST DOCTORAL FELLOW

An exciting opportunity exists for a post-doctoral fellowship to study the role of the hormone relaxin in the regulation of oxytocin secretion by the neurohypophysis. The work will involve *in vitro* techniques to explore the physiological processes involved. It will also contribute to a clinical study of the disease of lactating sows, known as hypogalactia. The work will be carried out in a well equipped laboratory which has other projects on relaxin in progress.

The successful candidate should have completed their doctoral thesis and have experience in tissue culture and organ perfusion techniques as well as in the handling, purification and assay of peptides. A general familiarity with reproductive biology would be an asset, although not essential.

The position will be available from April 1st, 1989. The stipend will be in the range of \$19,000 to \$24,000 and although the initial appointment would be for one year, there would be an opportunity for renewal for a further year given good progress.

Applications should be sent, together with a full curriculum vitae and the names and addresses of three referees, to **Dr. D. G. Porter, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G 2W1, Canada, (Tel. 519-824-4120, Ext. 4900)**, from whom additional information can be obtained.

*The University of Guelph is committed to Employment Equity* (NW3565)E

## POST-DOCTORAL FELLOWSHIPS IN MOLECULAR ONCOLOGY

Up to two positions will be available as early as 6/1/89 in the Department of Therapeutic Radiology at Yale University School of Medicine for young, innovative researchers interested in pursuing problems relevant to the molecular biology, diagnosis, and treatment of cancer. Projects include:

- 1) the development by genetic engineering techniques of new human hemoglobins able to improve delivery of oxygen to hypoxic tumor cells and their subsequent complete genetic, biochemical, and physiological characterization *in vitro* and *in vivo* (e.g., marrow transplant and transgenic mouse model systems), and;
- 2) the study of autocrine and paracrine mechanisms which govern stromal invasion by tumors, activated immune cells, and implanting embryos, with particular emphasis on the molecular biology of lymphohematopoietic cytokines, their receptors, and their biological role in the control of tumor growth, tissue invasion and metastasis, modulation of host response to foreign tumor and embryonic antigens, and alteration of tumor and normal tissue responses to radiation, chemotherapy, and other agents currently employed in cancer treatment.

Preference will be given to candidates with M.D. or Ph.D. degrees and research experience (pre- or post-doctoral) in molecular biology, protein and nucleic acid biochemistry, and/or molecular immunology. Interested individuals should send letters of application, including CV and references, to: **Barry M. Kacinski, M.D., Ph.D. 136 HRT, Dept of Therapeutic Radiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.** (NW3543)E

## STUDENTSHIPS



## DEPARTMENT OF GEOLOGY 1989 Triton (Europe) PhD Studentship

Applications are invited for a PhD research studentship in Geology to be based at the Department of Geology, University of Wales College of Cardiff and sponsored by Triton (Europe) PLC.

Funding will be over 3 years and at a level comparable to NERC awards but with a premium paid to the student on top of the maintenance grant.

The project will be sedimentary-based and involve regional studies of marine fan deposition. Interpretive Field investigations are planned in Southern France and these will be compared with analyses of seismic and borehole data in other areas.

Applicants who hold, or expect to obtain shortly a good Honours degree in Geology should write as soon as possible to:

**Professor R.B. Kidd, Department of Geology,  
University of Wales College of Cardiff,  
P.O. Box 914, Cardiff, CF1 3YE**

*Curriculum vitae and the names of two academic referees should be supplied.* (8920)F

## St Andrews University Department of Biochemistry and Microbiology POSTGRADUATE STUDENTSHIPS

Ph.D. studentships in the following areas are available in this well equipped and generously funded Department.

- |                           |   |
|---------------------------|---|
| <b>Dr. R.T. Hay</b>       | Role of sequence specific DNA binding proteins in viral replication and transcription                 |
| <b>Dr. G.D. Kemp</b>      | Structure function relationships in proteins involved in adenovirus infection                         |
| <b>Dr. R.E. Randall</b>   | Development of novel vaccination strategies in the prevention of viral disease                        |
| <b>Prof. W.C. Russell</b> | a) Adenovirus DNA binding protein b) Z DNA binding proteins   |
| <b>Dr. M.A. Mayo</b>      | Collaborative project (Scottish Crop Research Institute): molecular biology of potato leaf roll virus |
| <b>Dr. D. Thirkell</b>    | Analysis of the major antigens of Mycoplasma ovipneumonia   |
| <b>Dr. M.G. Burdon</b>    | Role of nickel in the Crease from Ureaplasma urealyticum  |
| <b>Dr. J.R. Kinghorn</b>  | a) Transformation system for Phytophthora infestans   |
|                           | b) Penicillin biosynthesis  |
| <b>Dr. J.L. Wray</b>      | Molecular genetics of sulphate metabolism and nitrate reduction in Barley                             |
| <b>Dr. W.J. Ingledew</b>  | a) Function and organisation of respiratory chain enzymes   |
|                           | b) Oxidation of Fe <sup>2+</sup> , pyrite and sulphur by Thiobacillus ferrooxidans                    |
| <b>Dr. R. Griffiths</b>   | Characterisation of acidic sulphur amino acids as endogenous neuroexcitatory transmitters             |

Potential candidates for admission can obtain further information by contacting **Dr. R.T. Hay, Department of Biochemistry and Microbiology, University of St. Andrews, North Street, St. Andrews, Fife, KY16 9AL, (tel. 0334 76161 ext 296).** (8908)F

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**UNIVERSITY OF  
SOUTHAMPTON**  
**CLINICAL PHARMACOLOGY  
GROUP AND MEDICINE  
RESEARCH  
STUDENTSHIP**

Applications are invited for a three year research studentship to commence in October 1989 researching into inflammatory factors causing human gastrointestinal diseases and drugs used in their treatment. The Group has excellent equipment and well funded projects. The student will receive broad training suitable for an industrial or academic career in a unit committed to high quality collaborative research. The University and surrounding area have excellent recreational facilities.

Applicants should hold or expect to obtain a good degree in the biological sciences.

Please send a full curriculum vitae and the names and addresses of two referees as soon as possible to **Dr K. Hillier, Clinical Pharmacology Group, Medical & Biological Sciences Building, Bassett Crescent East, Southampton SO9 3TU**, from whom further details can be obtained by telephoning 0703-595000, ext. 4264, or writing.

(8922)F

**DUNCAN GUTHRIE  
INSTITUTE OF  
MEDICAL GENETICS**  
(University of Glasgow)

**PhD Studentship**

A studentship leading to the degree of PhD is available from 1st October 1989. The studentship is funded by the Medical Research Council. The project will investigate molecular mechanisms regulating embryonic development in mice. Techniques to be used will include gene-cloning, cell culture, transgenic mice and *in situ* immunohistochemistry and hybridization histochemistry.

Further information may be obtained from **Dr. Rosemary Akhurst, Duncan Guthrie Institute of Medical Genetics, Yorkhill, Glasgow, G3 8SJ**. Tel. 041-339-6996, to whom applications should be made as soon as possible. (8912)F

**MEDICAL RESEARCH COUNCIL**

**National Institute for  
Medical Research**

**RESEARCH STUDENTSHIPS  
FOR OCTOBER 1989**

Applications are invited from students who hold or expect to obtain a first class or upper second degree, and are eligible for the following:

**MRC SCHOLARSHIPS**

**Dr. M. G. Sargent and Dr. P. D. Vize**

Gene products involved in the early stages of embryogenesis of *Xenopus*.

**Dr. F. Grosveld**

Characterization of the DNA binding proteins interacting with the dominant control region (DCR) of the beta-globin gene domain.

**Dr. P. M. Bayley**

The molecular mechanism of Calcium-activated cellular processes.

**Dr. D. R. Trentham**

Real time studies of cellular regulation by the second messenger, inositol trisphosphate (InsP<sub>3</sub>).

**Dr. M. R. Webb**

p21<sup>ras</sup> and cellular signal transduction.

**Dr. D. C. Ogden**

Regulation of ion channels by neurotransmitters.

**Dr. S. A. Wharton**

Receptor binding and membrane fusion interrelationships of influenza virus haemagglutinin.

**Dr. P. W. J. Rigby**

The spatial regulation of transcription during mouse embryogenesis.

**Dr. D. Wilkinson**

Role of zinc finger genes in segmentation in the mouse embryo.

**Dr. R. C. Hughes**

The role of carbohydrates and endogenous lectins in cell recognition.

**MRC COLLABORATIVE AWARDS WITH INDUSTRY**

**Dr. P. C. Emson** (at the Institute of Animal Physiology, Cambridge with **Dr. D. R. Hill** at MSD Research Laboratories, Harlow)

The pharmacology of central CCK expression.

**Dr. A. A. Holder** (with **Dr. D. Snary** of Wellcome Biotech. Beckenham)

Malaria parasite proteins involved in red cell invasion: the structure of a rhoptry protein and its role in inducing immunity.

**Dr. J. F. Eccleston** (with **Dr. P. N. Lowe** of Wellcome Research Laboratories Beckenham)

Structural studies on the interaction of the oncogene product, p21<sup>ras</sup>, with guanine nucleotides and GTPase activating protein.

**Dr. R. W. King** (with **Dr. D. G. Reid** of Smith Kline & French Research Ltd., Welwyn)

Binding of cAMP analogues to cAMP receptor protein.

**MRC AIDS DIRECTED PROGRAMME AWARD**

**Dr. A. J. Hay**

Elucidation of the ion-channel properties of the influenza virus M2 protein — the specific target of the anti-influenza drug, amantadine.

For further details and application form please contact **Dr. R. W. King, Director of Studies, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA**. Tel No 01 959 3666 extension 2279

**MRC**

(8903)F

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## Cancer Research Campaign

Fighting cancer on all fronts

### BEATSON INSTITUTE FOR CANCER RESEARCH Research Studentships in Cell Biology

Applications are invited for two Ph.D. Studentships from students expecting to graduate this year in science (first or upper second class honours degree), medicine or veterinary medicine.

Applicants should have a strong interest in cell biology and be prepared to learn techniques of molecular genetics. The projects relate to (1) interaction of retrovirus vectors with haemopoietic stem cells (AIDS directed MRC Studentship) and (2) cell-cell interactions in the control of proliferation and differentiation (CRC Studentship).

Further information may be obtained from Dr I. B. Pragnell (project 1) or Dr J. D. Pitts (project 2). Applicants interested in one or both projects should send a curriculum vitae and the names of two referees to **The Laboratory Manager (Ref. IP/JP), Beatson Institute for Cancer Research, Garscube Estate, Bearsden, Glasgow G61 1BD (041 942 9361) before 15th April.** (8914)F

## ASSISTANTSHIPS

### ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

#### DEPARTMENT OF VIROLOGY

### TWO POSTDOCTORAL SCIENTISTS ONE RESEARCH ASSISTANT

required to join expanding team investigating cytomegalovirus infections. Each post is for 3 years.

**POSTDOCTORAL POSITION ONE (ref. PD/RF)** will modulate the cell surface expression of Class I HLA molecules to determine if CMV can use these proteins as cellular receptors. Experience of recombinant DNA or immunological techniques desirable.

**POSTDOCTORAL POSITION TWO (ref. JG/RF)** will study the interaction between CMV and  $\beta_2$ -microglobulin to characterise, sequence, clone and express the viral proteins involved. Experience of the purification of proteins (not necessarily viral) from a complex mixture would be an asset.

**RESEARCH ASSISTANT (ref. SB/RA)** will participate in the studies of CMV,  $\beta_2$ -microglobulin and Class I interactions. Immunological experience desirable. Salary will be paid on Research Scales 1A/1B as appropriate plus £1,650 London Allowance. The Department is situated in modern purpose-built accommodation and is well equipped. These projects will provide ideal opportunities to broaden experience of modern virological techniques. Informal visits are welcome (tel. 01-794-0500 x3210 (Dr. Griffiths), x4118 (Dr. Grundy) or x4201 (Dr. Baldwin)).

Further particulars can be obtained from the **SCHOOL OFFICE (x4262), R.F.H.S.M., Rowland Hill Street, London NW3 2PF**, to which applications (*FOUR* copies of curriculum vitae including the names and addresses of two referees) should be sent by 21 April. (8930)P

### IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE (University of London)

#### Solid State Theory

### Postdoctoral Research Assistantships

Applications are invited for two Postdoctoral Research Assistantships to work with Professor D. Sherrington in the areas of (i) theory of complex disordered systems, including neural networks, and (ii) theory of high temperature superconductors. The posts, which are renewable, will be available from October 1 1989 and will be for one year in the first instance. Salary, which will depend on age and experience, will be on the PDRA A1 scale (currently £11,515 – £17,370 pa, inclusive of London Allowance).

The posts require experience in statistical or many-body physics. Candidates should send their CV and other relevant data to **Professor D. Sherrington, Blackett Laboratory, Imperial College, Prince Consort Road, London SW7 2BZ, UK, to arrive by 20 April 1989.** They should also arrange for two references to be supplied by the same date. (8931)P

### UNIVERSITY OF ST ANDREWS DEPARTMENT OF BIOLOGY AND PRECLINICAL MEDICINE POSTDOCTORAL RESEARCH ASSISTANTSHIP IN COMPARATIVE IMMUNOLOGY

Applications are invited for an SERC-funded postdoctoral research assistantship to investigate non-self recognition and complement activity in protochordates. The post is for two and a half years, commencing in early May or as soon as possible thereafter. Previous experience in biochemistry, immunology or microbiology is preferred. Starting salary will be within range £9,865 to £11,680 per annum, on 1A scale.

Further particulars and application forms can be obtained from the **Director of Personnel Services, The University, College Gate, St Andrews, Fife KY16 9AJ**, to whom applications should be sent to arrive *not later than 28th April 1989.* (8924)P

## SEMINARS & SYMPOSIA

### Update in Cardiopulmonary Research 23–27 October 1989

This five day symposium is organised by the Institute of Child Health, National Heart and Lung Institute, Papworth Hospital, The Royal Postgraduate Medical School and St George's Hospital Medical School. It will deal in depth with all the recent advances in this rapidly moving field.

#### Topics will include:—

- Congenital Malformations of the Heart
- Neural and Endocrine Control of Cardiovascular and Respiratory Systems
- Myocardial Ischaemia
- Pulmonary Circulation
- Heart and Lung Transplantations

#### Speakers will include:—

R H Anderson	T English	T Pexieder
L Allan	N Fagg	J M Polak
K M Allen	T Hallam	K Reimer
N Banner	S G Haworth	M N Sheppard
P Barnes	T Higenbottam	D R Springall
A E Becker	A Hislop	S Steward
G Burnstock	S Y Ho	P Taylor
D Clarke	A Khagani	J Tesh
C Clelland	M L Kirby	R P Thomson
S Cobbe	J Lundberg	J Wallwork
P Cummins	A Maseri	J Warren
F Cuttitta	W Martin	A C G Wenink
J Dark	R Mecham	J Wharton
E Dejana	S Moncada	T Williams
T A Dinh-Xuan	G Moscova	M Yacoub
M J Davies	J Pearson	

#### Venue:—

National Heart and Lung Institute  
Dovehouse Street  
London SW3 6LY

#### Registration fee:—

£200.00 — (or £50.00 for each individual day) includes catering but not accommodation

#### Further details available from:—

Postgraduate Centre  
National Heart and Lung Institute  
Dovehouse Street  
London SW3 6LY

#### Direct telephone line:—

01-351-8172

(8905)C

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## AMERICAN ASSOCIATION FOR CANCER RESEARCH

**EIGHTIETH ANNUAL MEETING  
SAN FRANCISCO, CALIFORNIA  
MAY 24-27, 1989**

**Over 2,500 Presentations of Recent Findings  
In All Areas of Cancer Research**

The Association welcomes non-members to its annual meeting. Young investigators are particularly encouraged to take advantage of this opportunity to hear and meet the leading scientists in the cancer field, to participate in workshops on important new laboratory techniques, and to take advantage of the Association's successful Employment Register.

### HIGHLIGHTS OF THE SCIENTIFIC PROGRAM SPECIAL LECTURES

Lawrence A. Loeb on molecular oncology  
Janet D. Rowley on molecular cytogenetics  
Raymond L. White on gene mapping and cloning  
Bert Vogelstein on genetic alterations  
Leonard J. Lerner on antiestrogens  
V. Craig Jordan on tamoxifen therapy

### SESSIONS OF INVITED SPEAKERS

(names of chairpersons in parenthesis)  
Hematopoietic growth factors (*Jerome E. Groopman*)  
Signal transduction and gene expression (*Ronald M. Evans*)  
Molecular and cellular approaches to proliferation and differentiation (*Gary S. Stein*)  
Molecular dosimetry and DNA repair (*Miriam C. Poirier*)  
Targets of anticancer drug action (*Kurt W. Kohn*)  
Immunoconjugates for cancer therapy (*Ralph A. Reisfeld*)  
Prediction of tumor response (*Sydney E. Salmon and Anne W. Hamburger*)  
Molecular aspects of growth regulation of breast cancer by hormones, growth factors, and oncogenes (*Geoffrey L. Greene*)  
New developments in tumors of the urothelial tract (*Alan Yagoda*)  
Novel approaches to drug dosing and scheduling (*Richard L. Schilsky*)  
Prospects for the medical control of the AIDS epidemic (*William A. Haseltine*)  
Epigenetics of cell transformation and tumor development (*Harry Rubin*)  
Role of glutathione S-transferase in drug resistance (*Cecil B. Pickett*)  
Lymphoid receptors and growth factors (*Cox Terhorst*)  
From epidemiology to cancer biology (*Frederick P. Li*)  
Molecular aspects of carcinogenesis (*Arthur P. Grollman*)

### METHODS WORKSHOPS

(names or organizers in parentheses)  
Cloning, expressing, and modifying genes and gene products (*Martin Rosenberg*)  
Transgenic mice as a model for oncogenesis (*Timothy A. Stewart*)  
The polymerase chain reaction: its subtleties and application (*Bernard J. Poiesz*)

### SESSIONS OF SUBMITTED PAPERS

Minisymposia and slide, poster, and poster discussion sessions will be scheduled on all four days of the meeting in all subfields of cancer research

### INFORMATION, REGISTRATION FORMS, AND MEMBERSHIP APPLICATION FORMS

available immediately upon request from:

**American Association for Cancer Research**  
530 Walnut Street, 10th Floor  
Philadelphia, PA 19106  
Telephone: 215-440-9300  
FAX: 215-440-9313

Information also available on the Association's new series of smaller, more focussed scientific meetings: **AACR SPECIAL CONFERENCES IN CANCER RESEARCH** (NW3548)C

## Molecular Communication in Higher Plants

18-21 September 1989  
at EMBL, Heidelberg, F. R. Germany

### Invited speakers include:

D. Baulcombe (GB-Norwich), M. Bennett (GB-Kew), M. Bevan (GB-Cambridge), T. Bisseling (NL-Wageningen), U. Bonas (D-Berlin), C. Bowler (B-Gent), F. de Bruijn (D-Köln), M. Caboche (F-Versailles), N.-H. Chua (USA-New York), A. Clarke (Australia-Melbourne), E. Coen (GB-Norwich), G. Coruzzi (USA-New York), J. Dangl (D-Köln), E. Dennis (Australia-Canberra), A. Gatenby (USA-Wilmington), W. Gerlach (Australia-Canberra), A. Gierl (D-Köln), R. Goldberg (USA-Los Angeles), W. Gruissem (USA-Berkeley), R. Hedrich (D-Göttingen), T. Hohn (CH-Basel), R. Horsch (USA-St. Louis), D. Jofuku (B-Gent), J. Jones (GB-Norwich), J. Leemans (B-Gent), P. Meyer (D-Köln), D. Mohnen (USA-Athens), T. Nelson (USA-New Haven), K. Palme (D-Köln), I. Potrykus (CH-Zürich), P. Quail (USA-Albany), E. Schäfer (D-Freiburg), A. Sievers (D-Bonn), C. Somerville (USA-East Lansing), A. Spina (D-Köln), D. van der Straeten (B-Gent), A. Trewavas (GB-Edinburgh), S. C. de Vries (NL-Wageningen), E. Weiler (D-Bochum), P. Weisbeek (NL-Utrecht), U. Wienand (D-Köln), L. Willmitzer (D-Berlin).

### There will be nine plenary sessions which will cover:

Cell-cell interactions; Differential gene expression/mutational analysis of plant function; Inter and intracellular signalling and the regulation of plant growth; Plant and environment; Plant biotechnology; Plant and microbes; Plants and viruses; Protein traffic and assembly into higher order structures; Structure, modification and expression of the nuclear genome (including novel techniques for gene isolation). The two poster sessions will allow participants to present their work.

### Registration:

The Symposium will be at the European Molecular Biology Laboratory, Heidelberg, with registration on Sunday, 17 September 1989. The registration fee, which includes daily transport to and from the EMBL, lunches, and the Symposium reception, is DM 180, for graduate students DM 90, and for participants from industry DM 360. Participants will be accommodated in the EMBL guest house and hotels in Heidelberg. The registration fee does NOT cover the cost of accommodation.

### Application:

The deadline for applicants is 16 June 1989. Applications should include a curriculum vitae and a brief description of research interests. The organizing committee will notify those who have been accepted as soon as possible after the deadline. The total number of participants will be limited to 250. Applications should be addressed to **Dr. J. Tooze, EMBO, Postfach 1022.40, D-6900 Heidelberg, F. R. Germany**. Applicants wishing to present a poster should send a 1-page abstract together with the registration fee and reply sheet after they have been accepted for participation.

### Organizing committee:

C. Leaver (Edinburgh) -Chairman-, K. Marcker (Aarhus), M. van Montagu (Ghent), I. Potrykus (Zürich), H. Saedler (Köln), F. Salamini (Köln), J. Tooze (EMBO, Heidelberg). (W5907)M

## COURSES & CONFERENCES

### ANNOUNCING AN NIH CONFERENCE

#### Modeling in Biomedical Research: An Assessment of Current and Potential Approaches

#### Applications to Studies in Cardiovascular/ Pulmonary Functions and Diabetes

To be held 1 to 3 May 1989, in Masur Auditorium, The Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland

Sponsored by the Division of Research Resources, the Division of Research Services, and the Office of Medical Applications of Research of NIH.

Continued innovation and refinement of model systems is crucial to rapid progress in biomedical research. This conference continues NIH's evaluation of modeling, from the use of non-human primates and invertebrate species to cell cultures and mathematical and physical approaches. To emphasize the use of many types of models to solve basic biomedical questions, the conference will focus on two areas of great importance to the nation's health: cardiovascular/pulmonary function (1 May 1989) and diabetes (2 May 1989).

Scheduled presenters on 1 May include: J. I. E. Hoffman, M. Gimbrone and F. Dewey, N. Staub, S. Factor and R. S. Chadwick, C. Peskin, S. Wickline, R. Ruffolo, and K. Brown and R. Robertson. Those on 2 May include: J. Roth, G. Grodsky, O. M. Rosen, D. Greene, A. Rossini, P. Lacy, R. Bergman, and J. Fain.

A panel of scientists chaired by Dr. Gordon H. Sato, Director of W. Alton Jones Cell Science Center, will question the presenters, prepare a summary statement of the material presented, and evaluate the strengths and limitations of the various model systems. On 3 May 1989, the panel will present its draft statement and invite comments from the audience.

To registrar for the conference or obtain further details, contact: **Susan Wallace, Prospect Associates, Suite 500, 1801 Rockville Pike, Rockville, MD 20852; telephone: 301-468-6555.** (NW3544)C



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- ★ PEG cell fusion
- ★ Electrofusion
- ★ Cloning
- ★ Screening systems
- ★ Antibody purification
- ★ Immuno-blotting
- ★ The effects of affinity/avidity in different assay systems
- ★ Discussion on some of the current uses including antibody engineering and clinical applications

### Course organisers:

M Ritter, H Ladyman, R Hargreaves

### Course fee (incl. catering)

**£300**

### Further details from:

Wolfson Conference Centre,  
Royal Postgraduate Medical School,  
Du Cane Road  
London W12 0NN

### Telephone:

01-740 3117

(8900)C

## EIGHTH CSH CONFERENCE ON CANCER CELLS REGULATION OF EUKARYOTIC mRNA TRANSCRIPTION



September 6 - 10, 1989

Organized by:

Winship Herr, Cold Spring Harbor Laboratory  
Robert Tjian, University of California, Berkeley  
Keith Yamamoto, University of California, San Francisco

The focus of this meeting is motivated by the rapid developments in the field of eukaryotic mRNA transcriptional control. The sessions will focus on:

- Initiation Complexes and Post-Initiation Events
- RNA Polymerase II and Basal Initiation Factors
- Mechanisms of Transcription Regulation: Activation Domains and Protein/Protein Interactions
- Structure and Function of Sequence-Specific DNA Binding Proteins
- The Influence of Template Topology, Nuclear Matrices, and Chromatin Structure on Transcription
- Tissue Selective Enhancer and Promoter Factors
- Regulation of Transcription Factor Activity
- Transcription of Developmentally Regulated Genes.

The meeting will include formal sessions with invited speakers and poster sessions. The organizers invite submission of abstracts. The abstract deadline is **JUNE 28, 1989.**

Kindly forward all forms and correspondence to:

**MEETINGS COORDINATOR  
COLD SPRING HARBOR LABORATORY  
COLD SPRING HARBOR, NY 11724  
516-367-8346 FAX 516-367-8845**

(NW3539)



# INTERNATIONAL CONFERENCE

## "THE GASTROINTESTINAL EPITHELIUM"

September 24-28, 1989,

RIOM, France

This conference is held under the auspices of the Institut National de la Santé et de la Recherche Médicale (INSERM) with the sponsorship of Merck Sharp Dohme-Chibret (France).

**Program:** Cell biology and molecular genetics of differentiation. Differentiation markers. New models for the study of GI epithelial cell differentiation. Maturation, growth and cell renewal. Cell renewal and carcinogenesis. Mechanisms and regulations of transport. Receptors. Interactions between the gastrointestinal epithelial cells and the immune system.

**Organising committee:** K. Haffen, P Hauri, M. Laburthe, M.J.M. Lewin, D. Swallow, G. Willems.

**Invited speakers:** D. Bataille, P. Burtin, J.F. Desjeux, B. Dutrillaux, S. Emami, R. Greig, D. Guy-Grand, D. Louvard, F. Martin, D. Ménard, M. Neutra, B.A.J. Ponder, F. Potet, J. Pouyssegur, J.C. Rambaud, F. Reyl-Desmars, G. Rosselin, G. Sachs, J. Schmitz, P. Simon-Assmann, A. Soumarmon, E. Wright, N.A. Wright, A. Zweibaum.

**Application:** A limited number of places is available for selected participants. Applicants should send a short CV and a brief outline of their research interests with 2 to 3 recent references, **before May 31 1989**, to M.J.M. Lewin (address below). Notification of acceptance will be mailed by June 15.

**Conference fee:** The selected participants will have to pay a registration fee of FF 1,000. Hotel accommodation and expenses will be covered by the Conference.

**Information and Applications to:** M.J.M. Lewin, "The GI Epithelium", INSERM U.10, Hop. Bichat, 75877 PARIS CEDEX 18, France.

Tel. (1) 40.25.83.90 & (1) 40.25.83.93. Fax (1) 46.27.85.36.

(W6035)C

THE ROBERT A. WELCH FOUNDATION CONFERENCE ON CHEMICAL RESEARCH

### XXXI. MEMBRANE PROTEINS: TARGETING AND TRANSDUCTION

Program Chairman: Joseph L. Goldstein

October 23 and 24, 1989

Houston, Texas

Speakers and Moderators\*

Michael Berridge	Daniel E. Koshland, Jr.*	Joseph Sambrook
Anter Blobel	Robert Lefkowitz	Gottfried Schatz
Erhard Bloch*	Yasutomi Nishizuka	Melvin Simon
Michael Brown	Shosaku Numa	Lubert Stryer
Fred Gilman	George E. Palade*	Don Wiley
Shigeharu Hanafusa	Hugh R. B. Pelham	
Ulfert Kornfeld	James Rothman	

Registration information will be published in August 1989. Contact: Kimberly Nelson, The Robert A. Welch Foundation, 4605 Post Oak Avenue, Suite 200, Houston, TX 77027, telephone 713-961-9884, FAX 13) 961-5168. (NW3569)C

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Second IIGB Meeting

### Workshop on Molecular Biology of Development

2-4 October 1989, Capri, Italy

Organizers:

Paolo Bazzicalupo, Franco Graziani,  
Edoardo Boncinelli, M. Graziella Persico (IIGB, Naples)

Topics cover:

Gene expression in developing systems as exemplified by  
*D. melanogaster*, *C. elegans*, *X. laevis* and mouse.

Invited speakers include:

M. Ashburner, Cambridge	R. Horvitz, Cambridge, Mass
J.A. Campos-Ortega, Köln	D. Ish-Horowicz, Oxford
M. Chalfie, New York	H. Jaekle, München
T.W. Cline, Princeton	R. Krumlauf, London
I. Dawid, Bethesda	M. Levine, New York
E.M. De Robertis, Los Angeles	A. Martinez-Arias, Cambridge
D. Duboule, Heidelberg	W.J. McGinnis, New Haven
A. Garcia-Bellido, Madrid	D.A. Melton, Cambridge, Mass.
W.J. Gehring, Basel	M. Noll, Basel
P. Gruss, Göttingen	C. Nüsslein-Volhard, Tübingen
J.B. Gurdon, Cambridge	M. Rosbash, Waltham
D. Hirsh, Boulder	A.C. Spradling, Baltimore
J. Hodgkin, Cambridge	C. Wu, Bethesda

The number of participants is limited to 70. Applications, together with a curriculum vitae and a list of research interests, should be sent to Dr. Edoardo Boncinelli, IIGB, Via Marconi 10, 80125 Naples, Italy, before 1st July 1989. A registration fee of US \$ 200 will include board and lodging. Successful applicants will be notified by the end of August 1989.

(W6027)V

First announcement of a workshop on:

# POST-TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION

at the Gesellschaft für Biotechnologische Forschung mbH,  
Braunschweig, West-Germany, April 11 - 16, 1990.

Topics to be covered (in prokaryotic and eukaryotic systems):

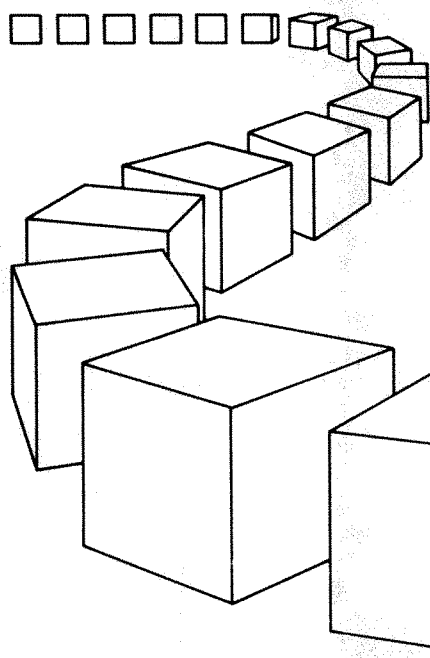
1. Control of mRNA stability
2. Roles of antisense RNA
3. Transcriptional (anti-) termination
4. Control/regulation of translation
5. Translational fidelity (including frameshifting)

A list of speakers will be published in the near future. A limited number of places have been left free for further applicants, who should apply to either of the organisers:

Dr. J.E.G. McCarthy  
Gesellschaft für Biotechnologische  
Forschung mbH  
Mascheroder Weg 1  
D-3300 Braunschweig  
West Germany

Dr. M.F. Tuite  
Biological Laboratory  
University of Kent  
Canterbury  
Kent CT2 7NJ  
UK

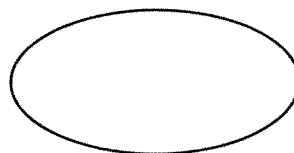
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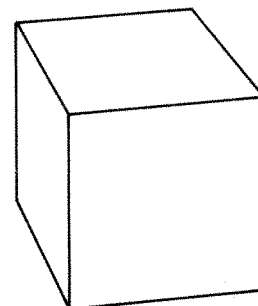
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2nd INTERNATIONAL CONFERENCE

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| R. BRAVO      | Identification of growth factor inducible genes  |
| P. CHAMBON    | Nuclear receptors as inducible enhancers   |
| T. CURRAN     | Beyond the second messenger: Fos, Jun and the AP-1 binding site  |
| R. EISENMAN   | What information do Myc and Myb carry to the nucleus?  |
| E. GATEFF     | Differentiation and tumor suppressor genes in <i>Drosophila</i>  |
| E. HARLOW     | The retinoblastoma protein is a common target for transformation by DNA tumor viruses  |
| D. HOUSMAN    | Inactivation of the c-kit locus in mouse w mutants   |
| E. KANDEL     | Cell and molecular biological approaches to long-term memory   |
| H. LAND       | Oncogene cooperation <i>in vivo</i> and <i>in vitro</i>  |
| J. MALLER     | Molecular elucidation of the S6 kinase pathway   |
| K. MATSUMOTO  | Signal transduction in yeast: mating pheromone and RAS-cAMP pathways   |
| F. McCORMICK  | The role of GAP and its interaction with ras in growth signal cascades   |
| S. McKNIGHT   | Molecular aspects of regulation by cAMP kinase   |
| S.L. McKNIGHT | Unusual properties of a regulatory protein that facilitates differentiation in a coordinated network of mammalian cell types |
| W. MOOLENAAR  | Signalling pathways in the action of growth factors  |
| E. NIGG       | Signal transduction from cytoplasm to nucleus: A role for translocating kinases and shuttling substrates?                    |
| P. NURSE      | Cell cycle control in yeast – implications for mammalian cells   |
| R. NUSSE      | The <i>int</i> oncogenes in mammary tumorigenesis and in embryogenesis   |
| P. PARKER     | The protein kinase C pathway   |
| L. PHILIPSON  | Negative regulation of growth in mammalian cells   |
| G. SPRAGUE    | Cell type and cell communication in yeast  |
| K. TATCHELL   | Does cAMP regulate cell growth in yeast?   |
| W. TIMBERLAKE | Hierarchy hegemony and hysteresis in <i>Aspergillus</i> development  |
| J. THORNER    | Molecular genetics of calcium signalling in yeast growth control   |
| R. TJIAN      | Functional dissection of eukaryotic transcription factors  |
| N. TONKS      | Protein tyrosine phosphatases; structure, properties and involvement in signal transduction                                  |
| R. TREISMAN   | Isolation and properties of cDNA clones encoding the transcription factor SRF  |
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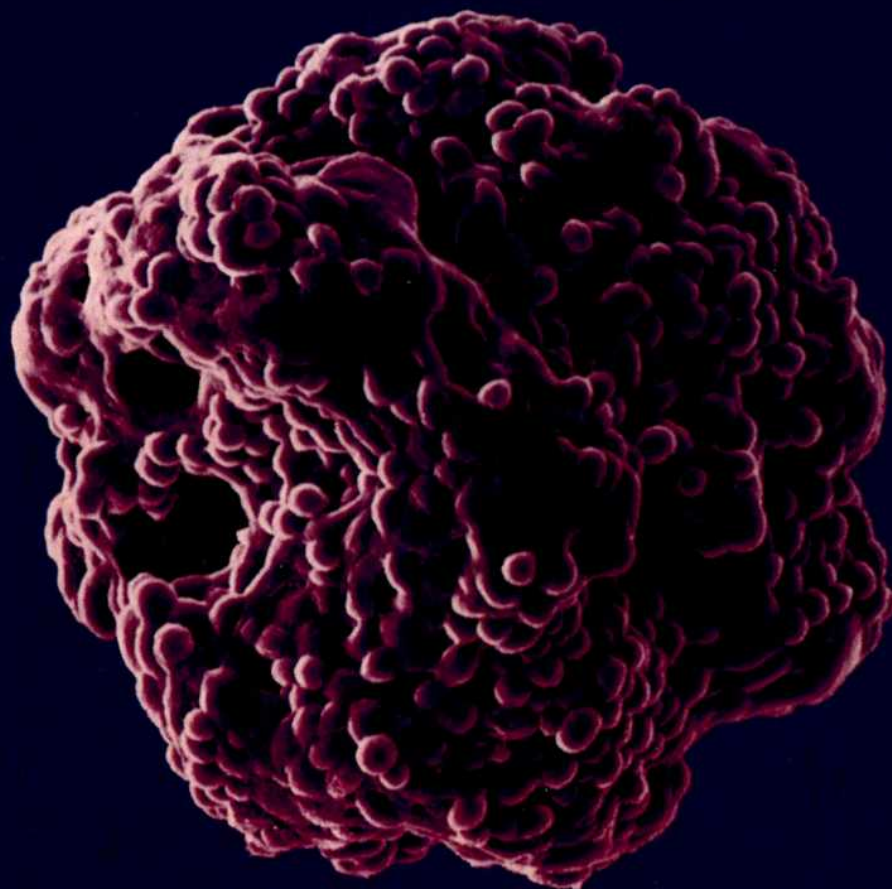
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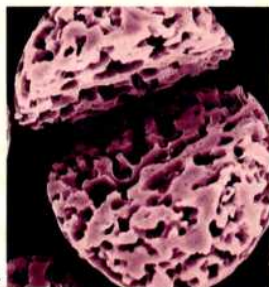
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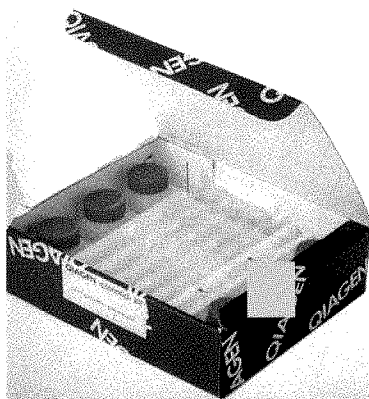
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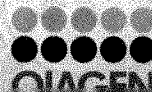
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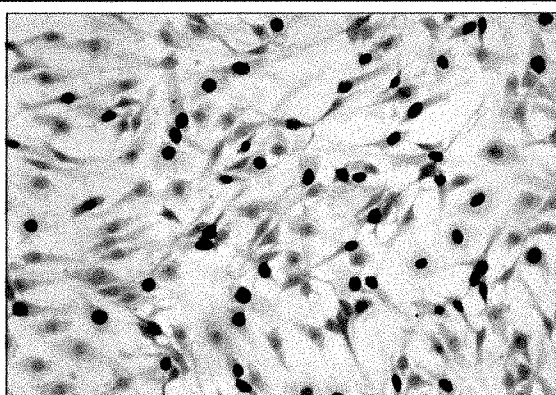
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1) Gratzner *et al*, *Science*, **218**, p.474, 1982

2) Campana *et al*, *J. Immunol. Methods*, **107**, p.79, 1988



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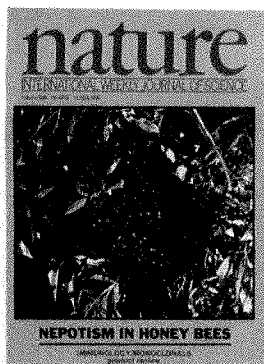
C6 glial cells. Cell proliferation demonstrated using the Amersham kit.

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# nature

13 April 1989

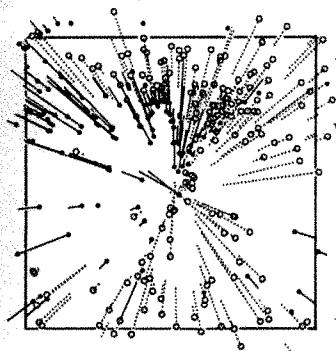
Vol. 338 Issue no. 6216

*Homo sapiens* is not the only species in which nepotism can be demonstrated — honey bee workers prefer to raise queens closely related to themselves. See page 576. Cover photo of a reproductive swarm by N. E. Gary, Department of Entomology, University of California at Davis.

## THIS WEEK

### The main attraction

The large-scale motions in the Universe can be explained by the presence of a 'Great Attractor', with a mass of more than  $5 \times 10^{16}$  times that of the Sun. A survey of galactic redshifts points to a



remarkable concentration of clusters as the origin of the attractive force. Pages 562 and 538.

### Bio-semiconductors?

The peptide-coated cadmium sulphide crystallites produced in some yeasts exposed to cadmium behave as true clusters, with quantum properties such as size-dependent electronic transitions. Page 596. See also page 540.

### Genes in clover

A pea lectin gene expressed in the roots of clover plants allows nodulation by a strain of *Rhizobium* normally specific for pea plants, supporting the idea that lectins may be important determinants of the host specificity of nitrogen-fixing bacteria. Pages 579 and 545.

### Insulin unfolds

The structure of a new crystalline form of hexameric insulin shows how the interaction of proteins with small molecules can affect their folding. Page 594.

### Palaeoclimate

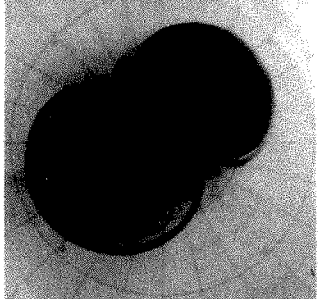
Abrupt changes in the climatic conditions of the North Atlantic during the past 18,000 years may have been caused by the gradual effects of the astronomical forcing regulating the incidence of ice ages, accentuated by the melting of the Laurentide Ice Sheet. Page 553.

### Third GABA subunit

Benzodiazepines regulate GABA receptors, but the two GABA receptor subunits cloned previously do not bind benzodiazepines. A third related subunit has now been cloned that confers a high affinity for benzodiazepines when coexpressed with the other two subunits. Page 582.

### Three-pole fluids

This tripolar pattern (below) has not yet been observed in the



world's oceans, but it is stable in the laboratory, page 569.

### Cell growth factor

Platelet-derived endothelial growth factor has a gene sequence unrelated to that of any characterized protein. Page 557.

### Diabetes risk

A specific allele within the major histocompatibility complex, DQ2, may contribute to susceptibility to insulin-dependent diabetes mellitus. Page 587.

### Guide to Authors

See page 598.

## NATURE SAYS

Mr Gorbachev's visit to London has sharpened the issue of tactical nuclear weapons ■ There are drawbacks to publishing discoveries first in newspapers ■ The Swiss may create a European university

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## NATURE REPORTS

Cold fusion still burns ■ Soviet sub sinks ■ UNIX 'worm' dissected ■ Virus in India ■ Britain in the Arctic? ■ Rain forest statistics disputed ■ Atmosphere authority ■ Japan's sales tax ■ Funds for joint research ■ Vet schools ■ Alaska oil spill ■ Future for French research ■ Laws for animals ■ Exploring Gorbachev ■ Greenhouse research

Down Under

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The creator's final word ■ Intuitive science ■

Quark history lesson

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Lymphocyte differentiation: Not all in a name

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Nine hundred kiwis and a dog

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Nitrogen fixation: New route to a sticky subject

Sharon R Long & David W Ehrhardt

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## SCIENTIFIC CORRESPONDENCE

Leucine zipper motif extends R Buckland & F Wild ■

Stereo problem B M Millman ■ Stop taxonomists

J A Barnett ■ Carbonatite origin and diversity

B Kjarsgaard & D L Hamilton; Reply—J Griffiths

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## BOOK REVIEWS

Methodological Aspects of the Development of Low

Temperature Physics 1881-1956: Concepts out of

Context(s) by K Gavroglu & Y Goudaroulis

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Introduction to Quasicrystals M V Jarić ed

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Introduction to the Modern Theory of Metals by A Cottrell

D G Pettifor

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Digging Dinosaurs by J R Horner & J Gorman

Leonard Krishtalka

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## ARTICLES

Climate change in the circum-North Atlantic region during the last deglaciation

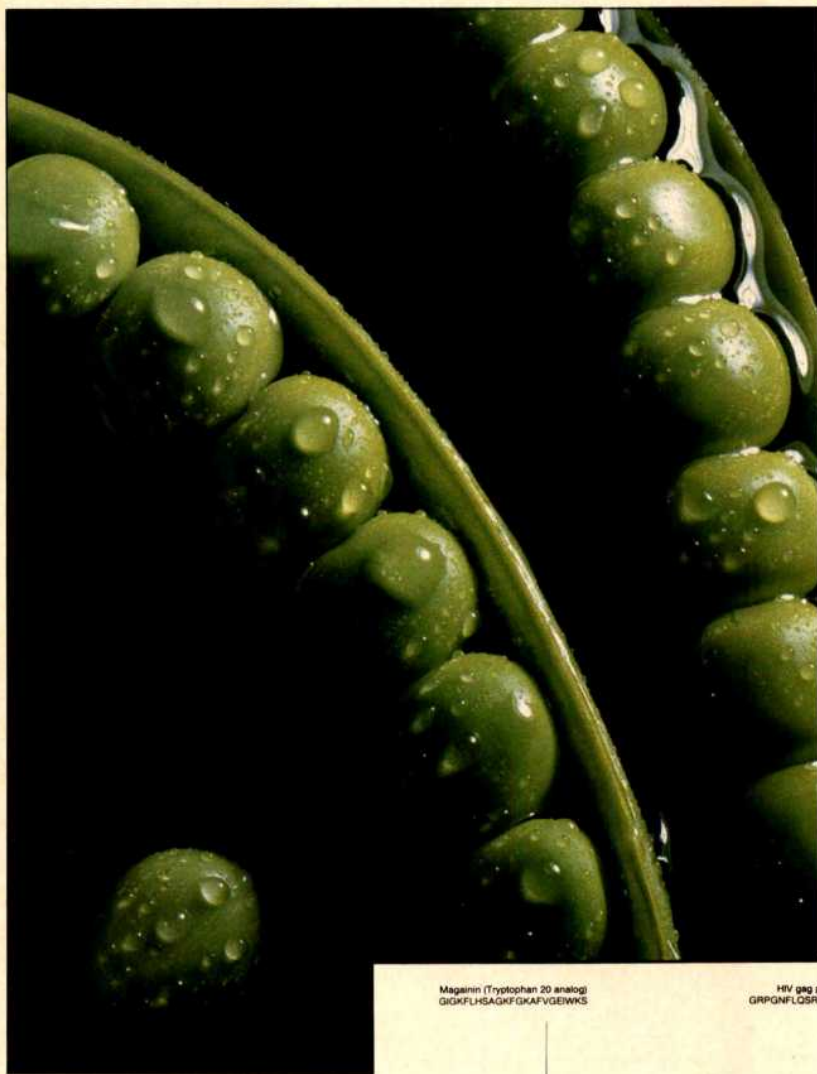
J T Overpeck, L C Peterson, N Kipp, J Imbrie & D Rind

553 ►

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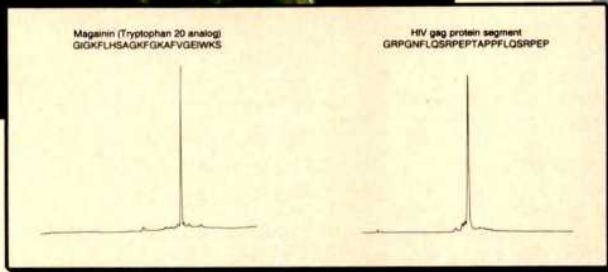
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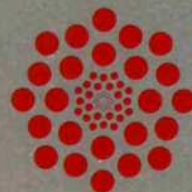
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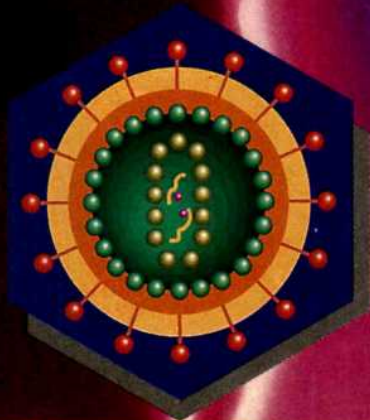




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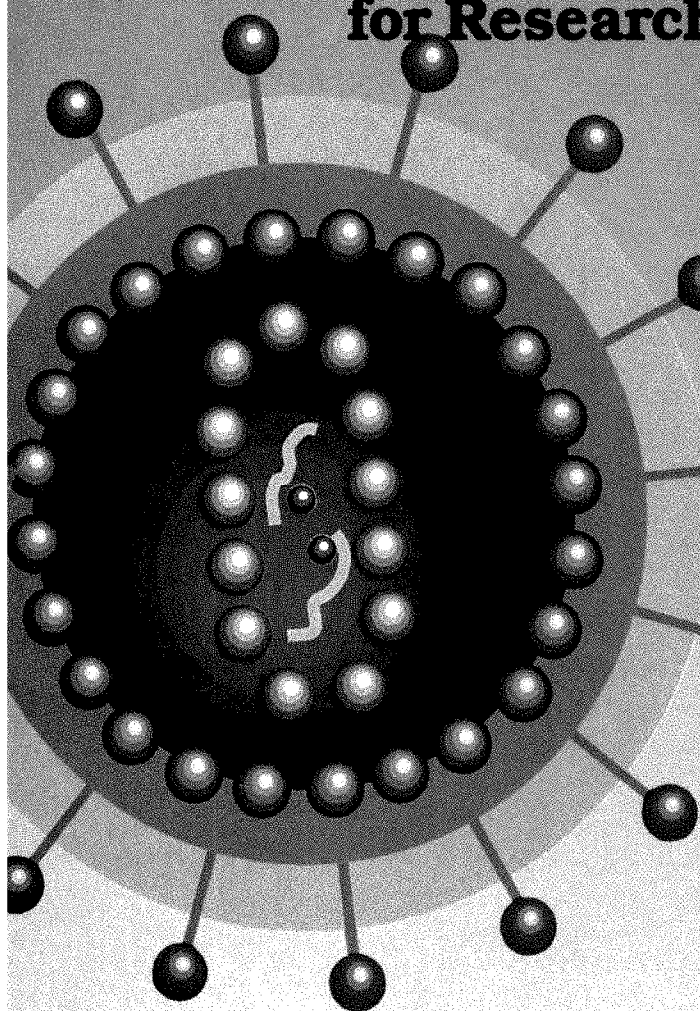
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ARG-VAL-VAL-GLN-ARG-GLU-LYS-ARG-OH  
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6120  
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gp41  
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H-GLY-CYS-SER-GLY-LYS-LEU-ILE-CYS-  
THR-THR-ALA-VAL-PRO-TRP-ASN-ALA-  
SER-OH  
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excellent antigen.

6130  
HIV-1  
gp41  
\$600/mg  
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H-ARG-ILE-LEU-ALA-VAL-GLU-ARG-TYR-  
LEU-LYS-ASP-GLN-GLN-LEU-LEU-GLY-ILE-  
TRP-GLY-CYS-SER-GLY-LYS-LEU-ILE-CYS-  
THR-THR-ALA-VAL-PRO-TRP-ASN-ALA-  
SER-OH  
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gp41 protein and was found to detect 148 out  
of 149 HIV-1 positive sera.

6140  
HIV-1  
gp41  
\$700/mg  
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H-ARG-ILE-LEU-ALA-VAL-GLU-ARG-TYR-  
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TRP-GLY-CYS-SER-GLY-LYS-LEU-ILE-CYS-  
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6210  
HIV-2  
gp41  
\$500/mg  
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TRP-GLY-CYS-ALA-PHE-ARG-GLN-VAL-CYS-  
NH2  
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with high sensitivity.

6220  
HIV-2  
gp41  
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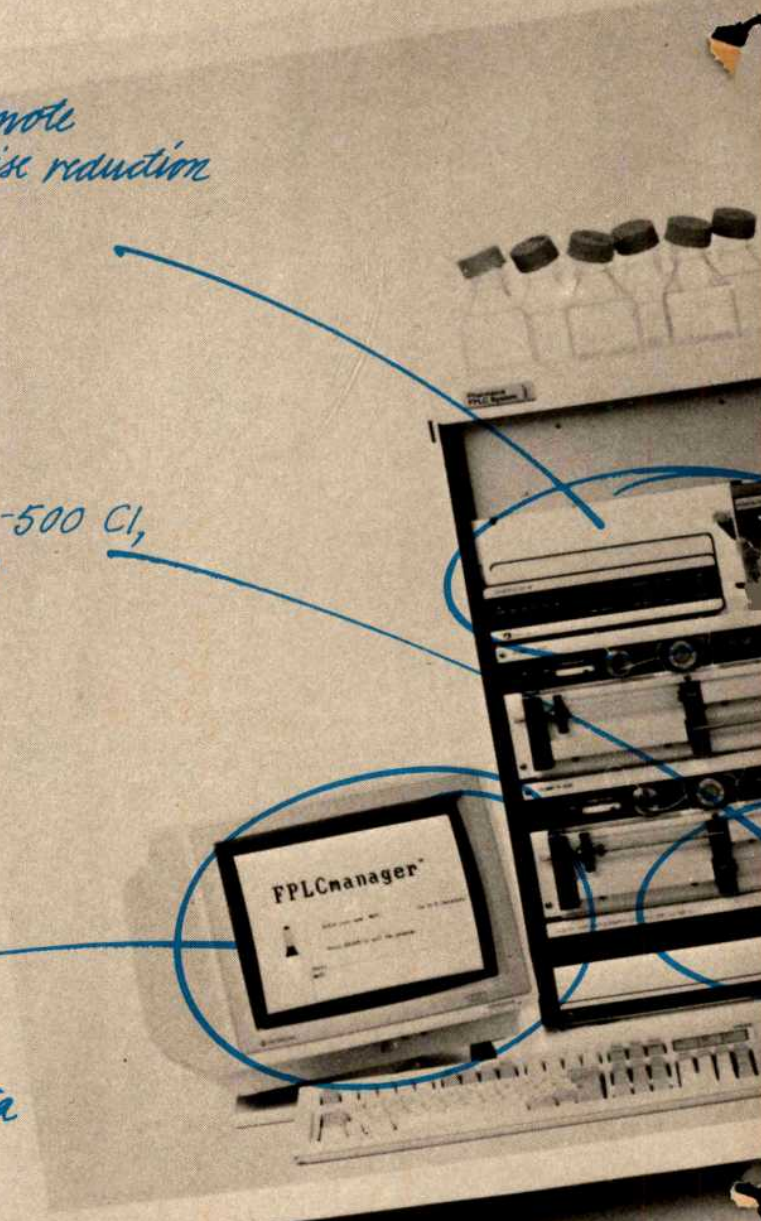
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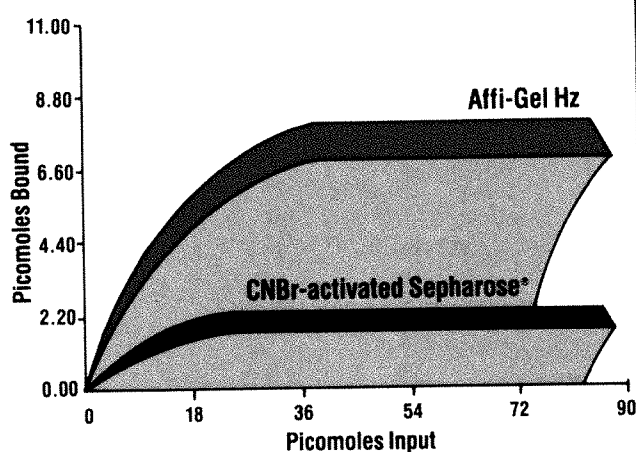
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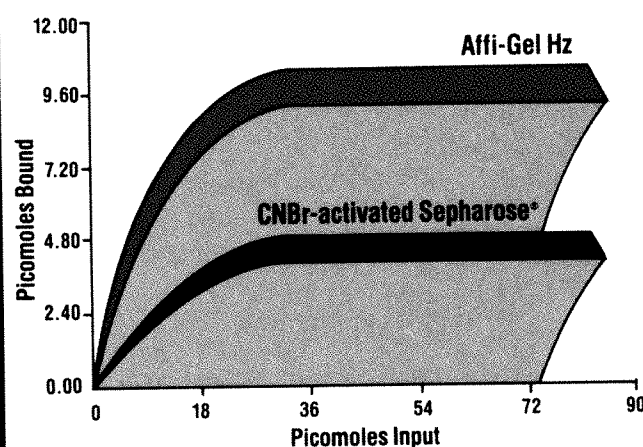


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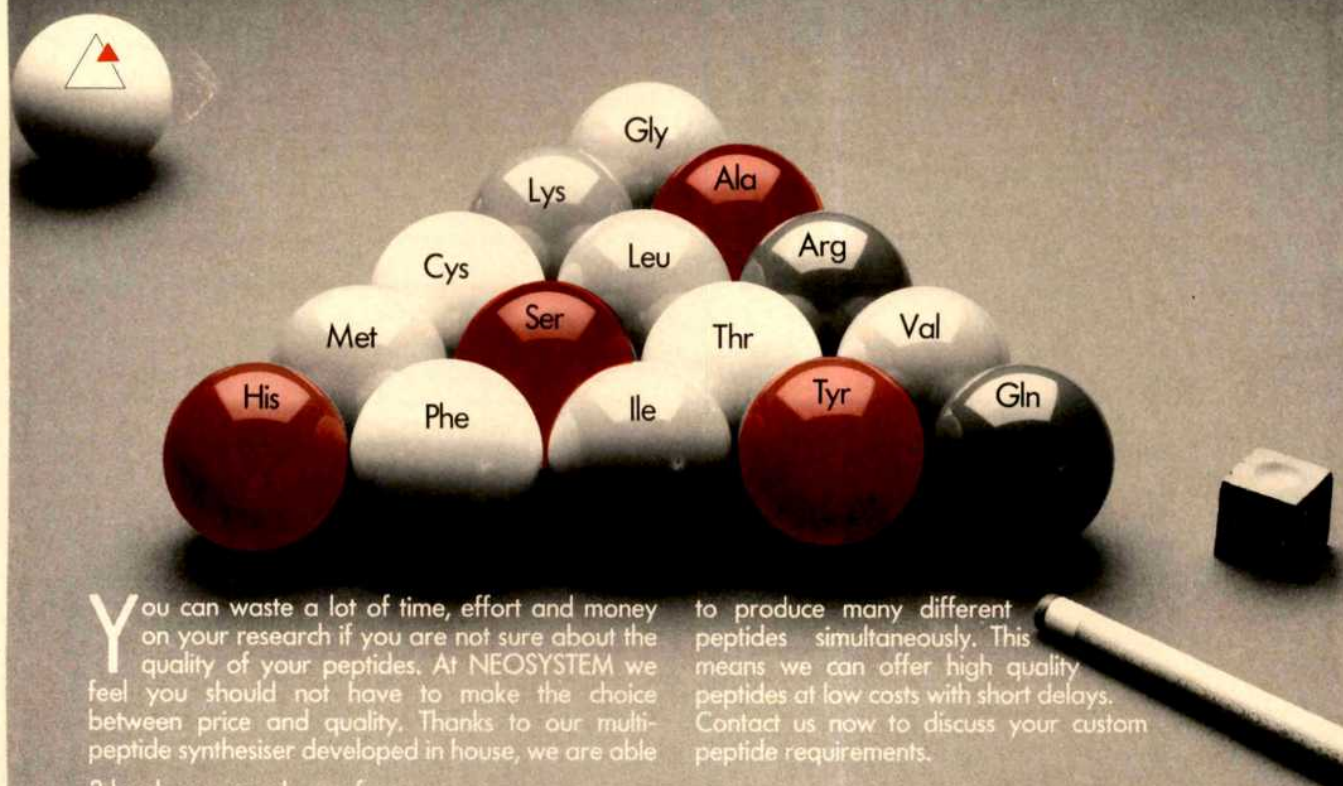
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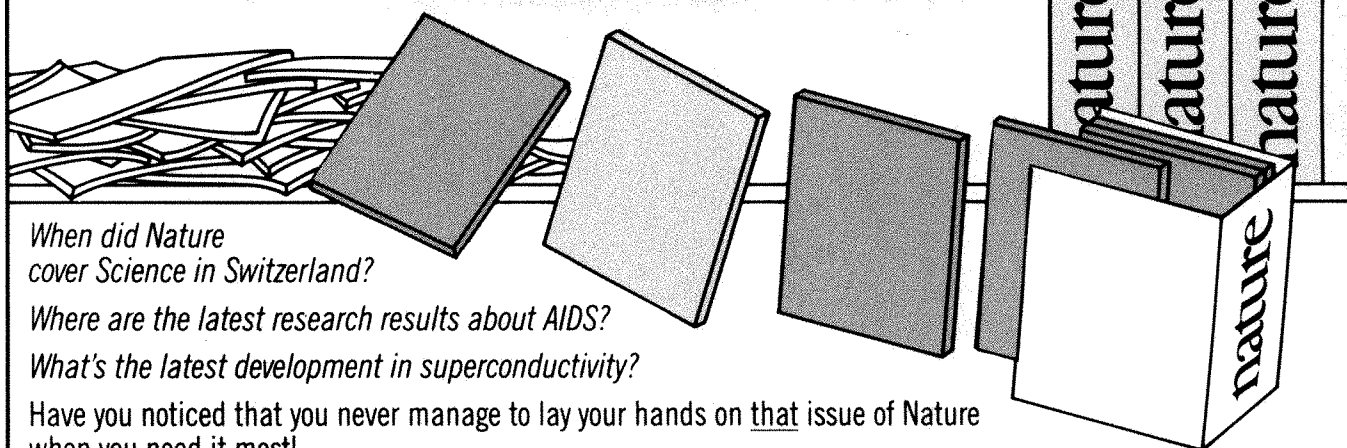
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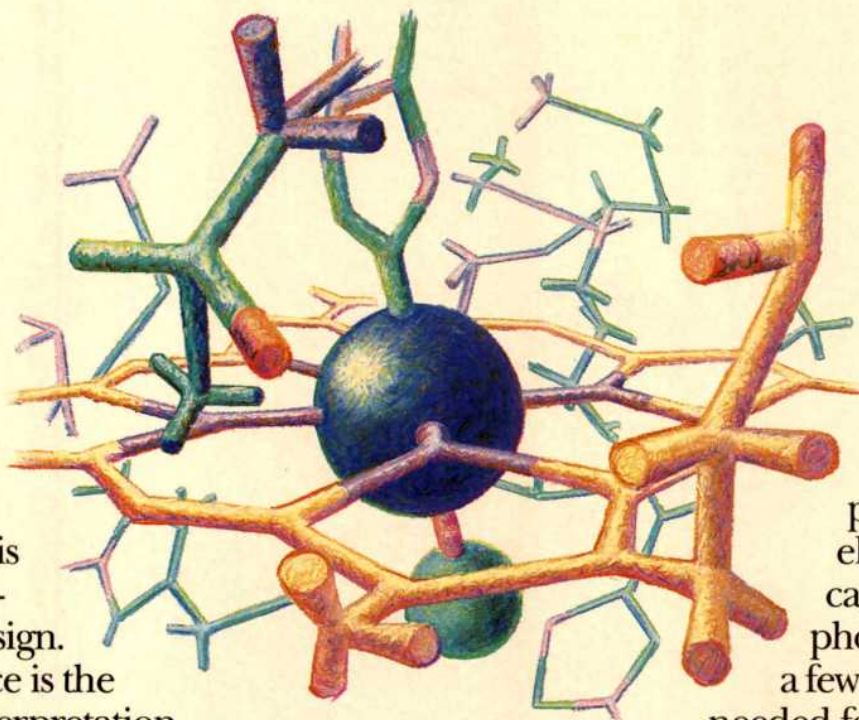


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# Is protein chemistry an art or a science?



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of protein  
chemistry is  
the experi-  
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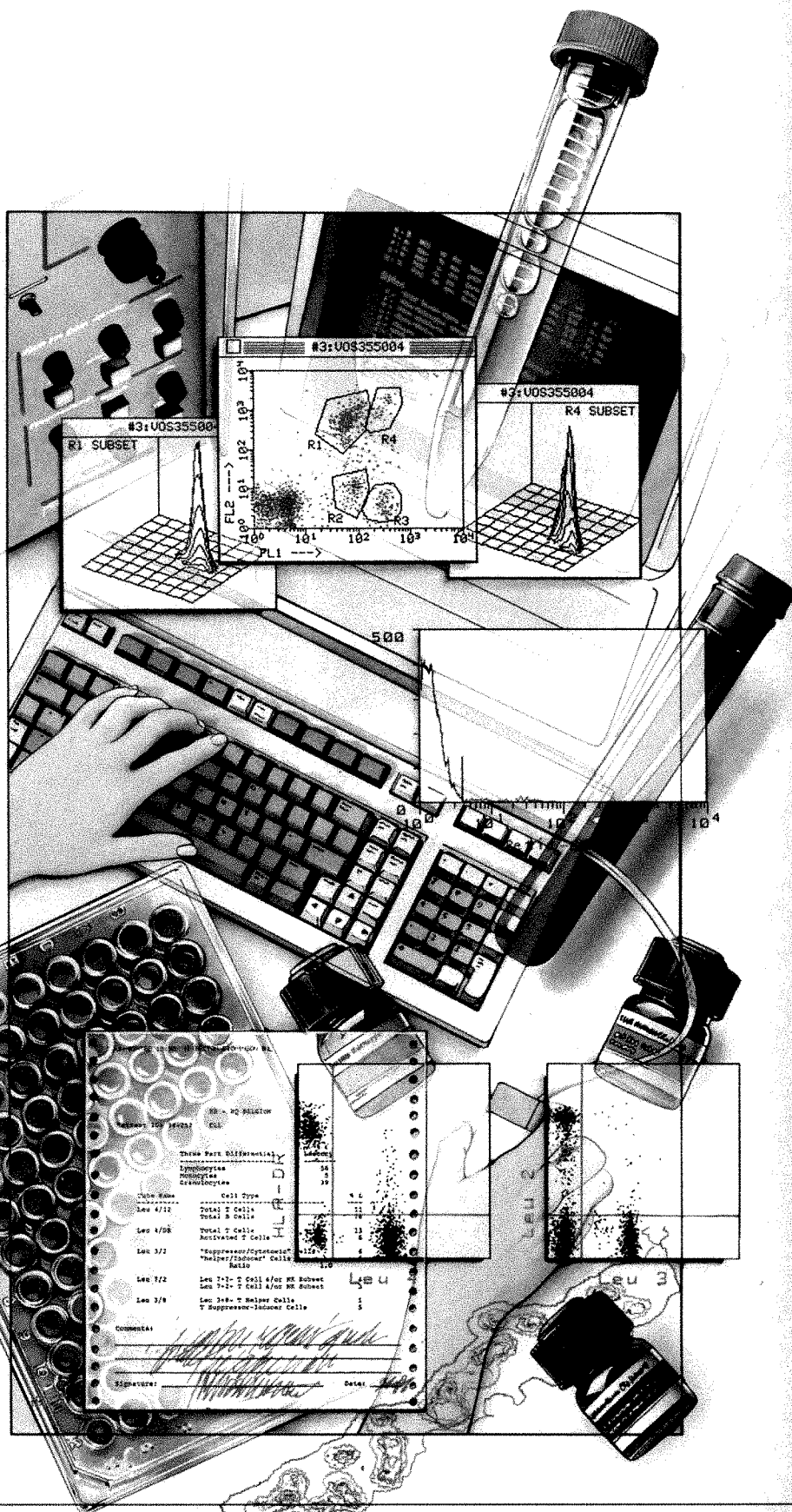
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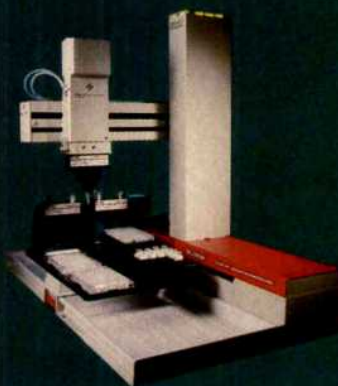


Figure 1

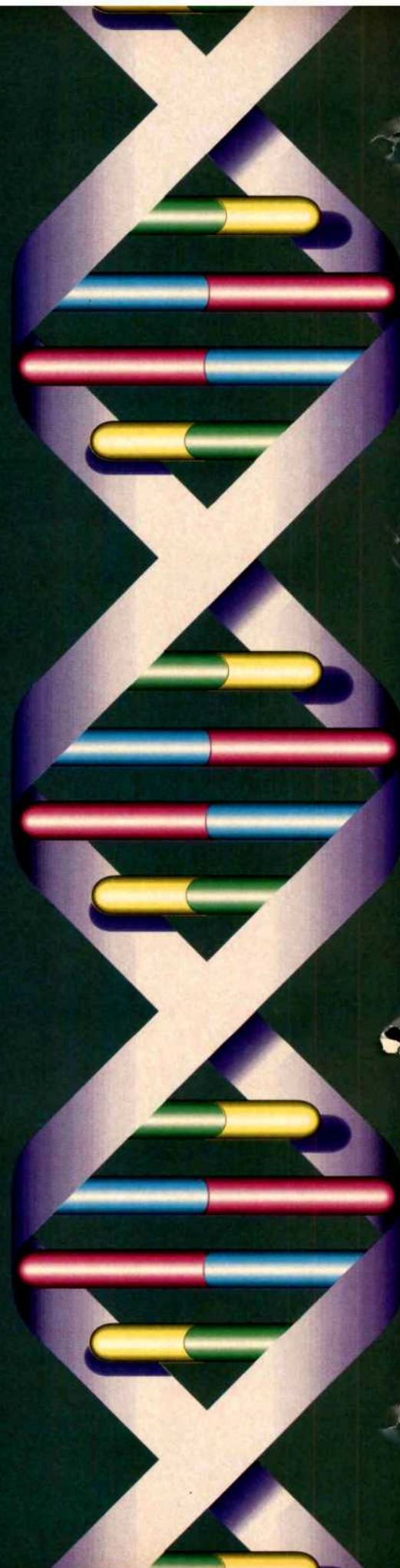


Figure 2



Figure 1. Autoradiogram of sequence analyses accomplished on the Biomek 1000 using GemSeq K/RT double-stranded DNA sequencing system and Riboprobe Gemini pGEM-3 vector, containing an insert of a known cDNA.

Figure 2. Autoradiogram of sequence analyses using M13mp18. Lanes 1-9 were performed manually, lanes 10-18 were performed on the Biomek 1000.





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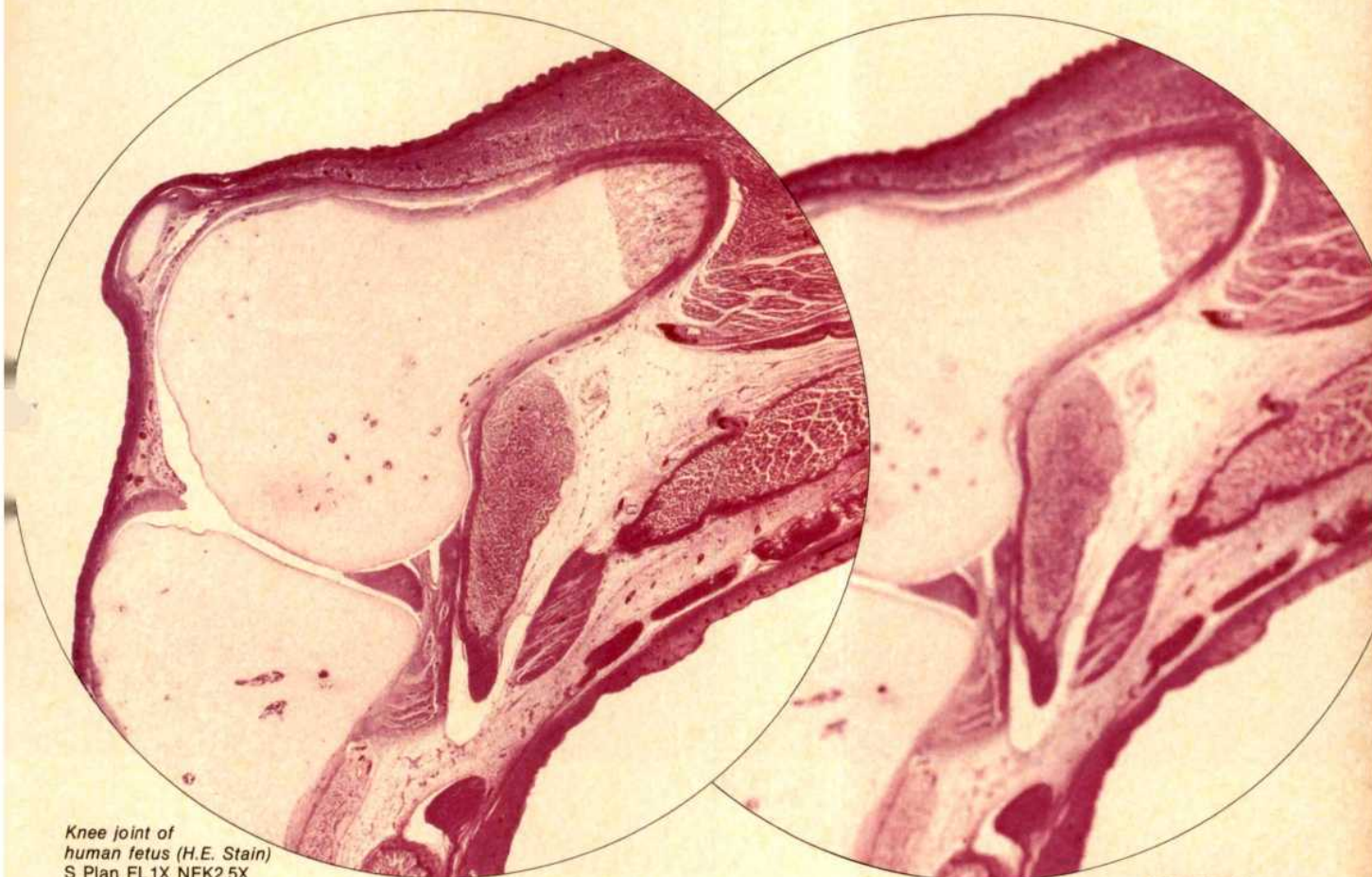
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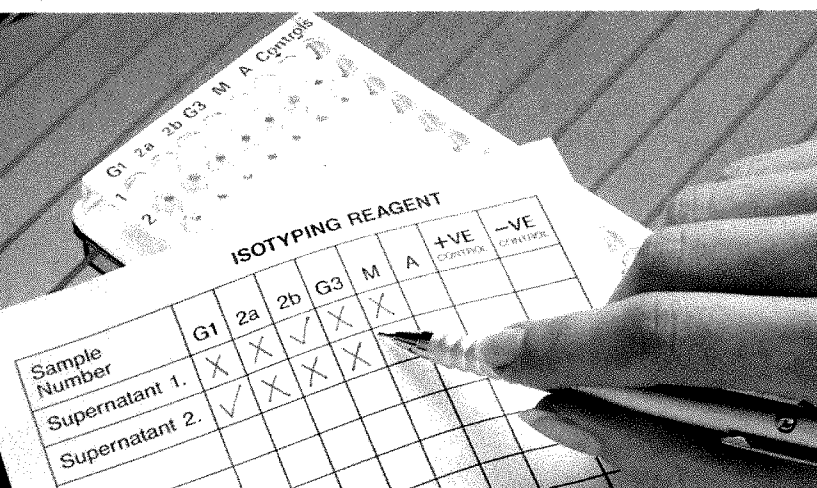


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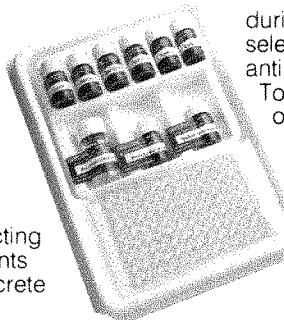
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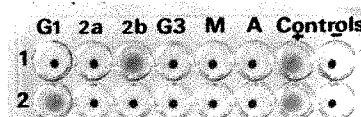
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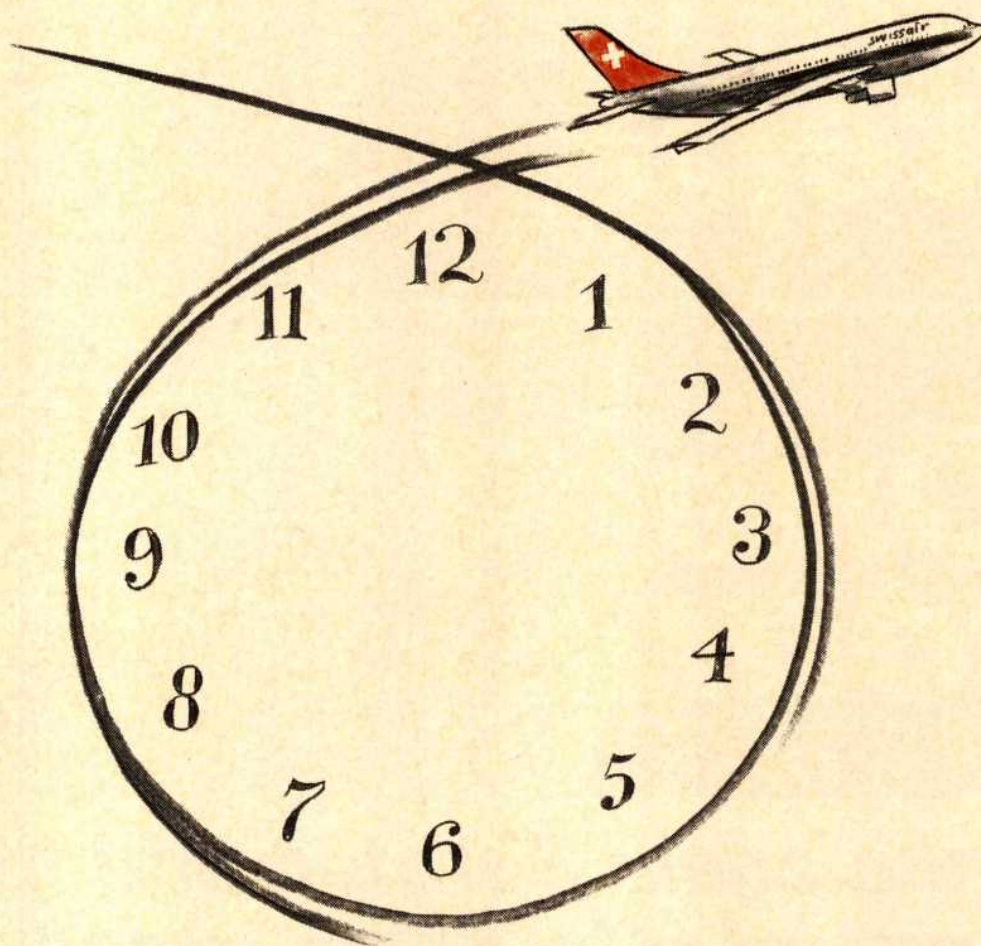
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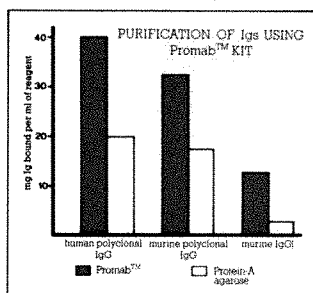
Product Code	Glycine Buffer pH9.6 25 C Biozyme Assay U/mg	Glycine Buffer pH10.4 37 C U/mg	Diethanolamine Buffer pH9.8 37 C U/mg
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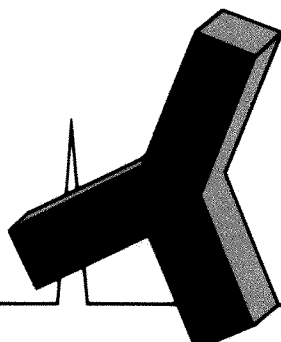
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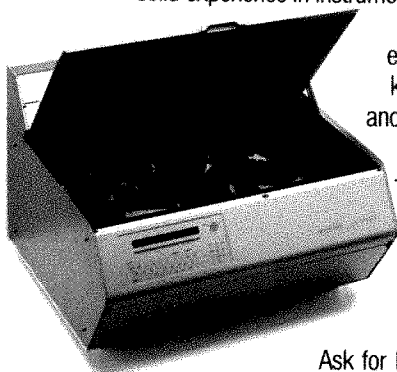
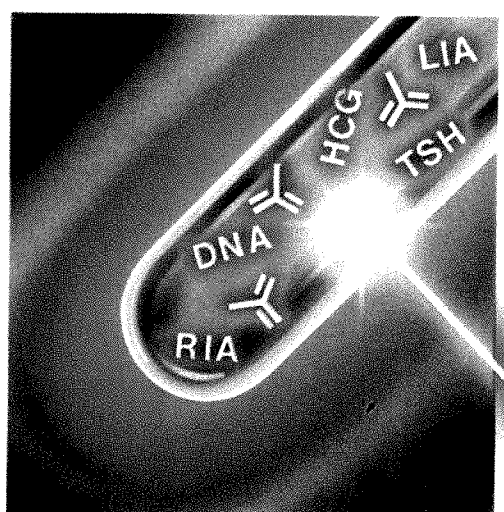
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\*This instrument has successfully been used to transfer genes into mammalian cells (T- & B-lymphocytes, hepatocytes, fibroblasts, kidney & neural cells, etc.), plant cells (monocot & dicot, both intact cells and protoplasts) and bacterial cells (Gram positive & negative). It also has been used to induce cell fusion among mammalian, fish, plant, and bacterial cells.

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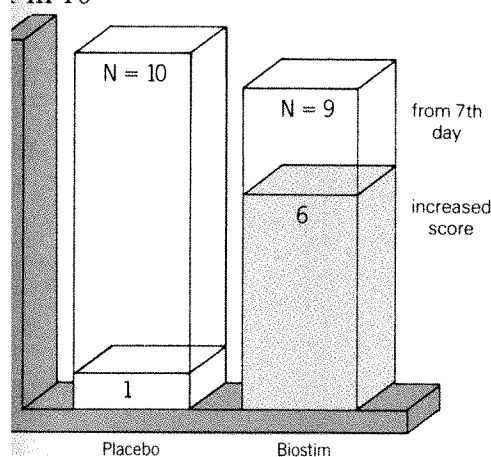
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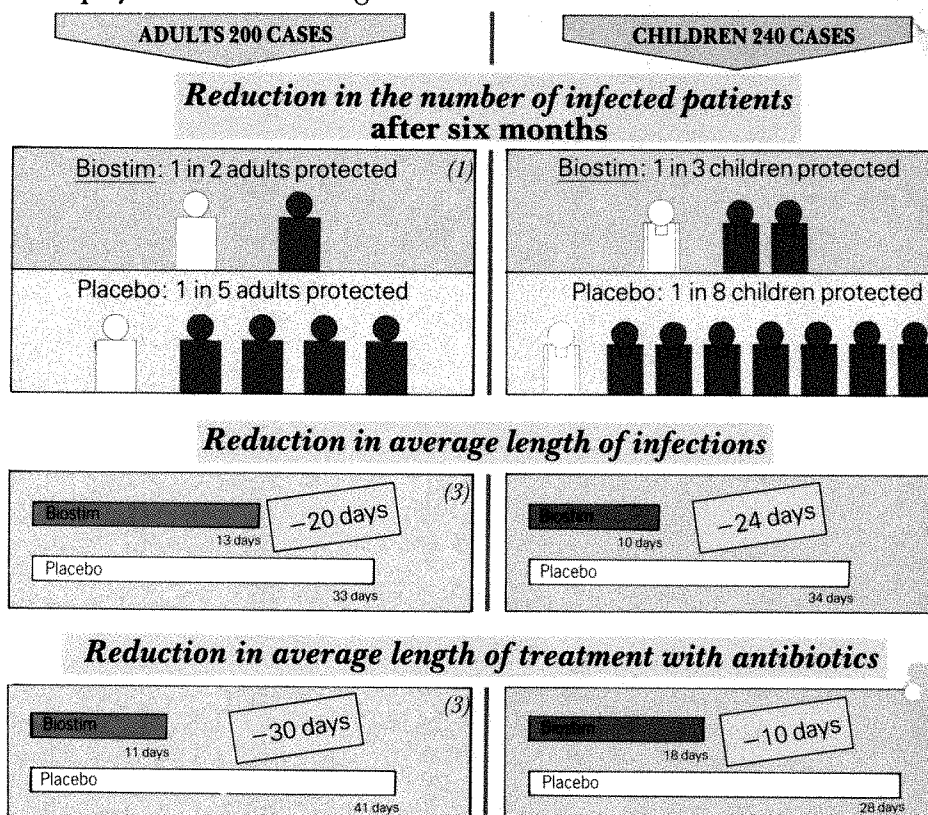
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form and presentation. Coated tablets (white) in blister packing, box of eight. Ingredients. Weight of ingredients 1 mg. Weight of box 8 mg. Glycoproteins extracted from *Beauveria pseudocillata* retained on a membrane of average porosity 0.011 micrometres and expressed in anhydrous product. Excipient: lactose, corn starch, glycidol, magnesium stearate, cellulose, croscarmellose, sodium carboxymethylcellulose, colloidal silicon, talcum, polysorbate 80, titanium dioxide, circelanche q. s. p. per 400mg tablet. Properties. (cp. monograph by Vidal). BioSTIM is an immunostimulant. Tests have shown it to reactivate all three levels of

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(1) Prof. Chrétien 52 cases — *Ref. Rev. Pneumol. Clin.*, 1985, 41, 3: 213-217

(2) Prof. Pech 78 cases — *Ref. Cahiers ORL*, 1987, XXII, 3: 217-220

(3) Prof. Boutin 73 cases — Study over 9 months, *Ref. Poumon Coeur*, 1983, 39: 53-57.

(4) Prof. Paupe 42 cases — Study over 6 months *Ref. Ann. Pédiatrie*, 1986, 33 (9): 843-845

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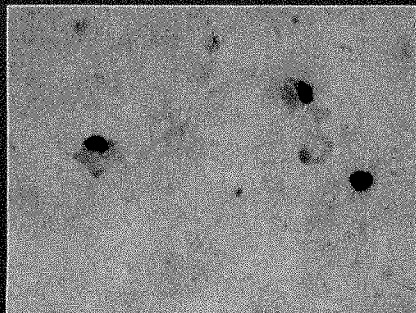
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(Volkers, van den Brink & Houthoff, Amsterdam)

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(van Lookeren-Campagne & van Bergen en Henegouwen, Utrecht)

← Blot of total extract of Hela cells and blot of partially purified nuclei of rat liver reacted respectively with rabbit anti-tubulin (a) and rabbit anti-actin (c) followed by AuroProbe One and IntenSE. Negative controls (b, d)

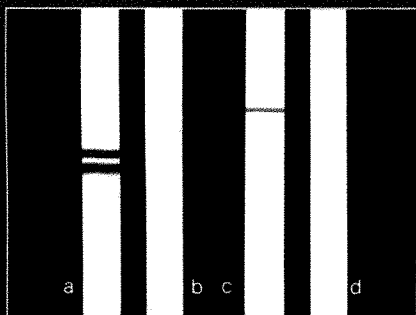
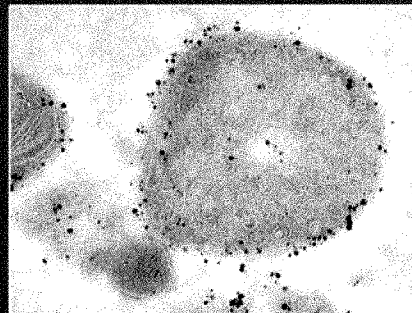
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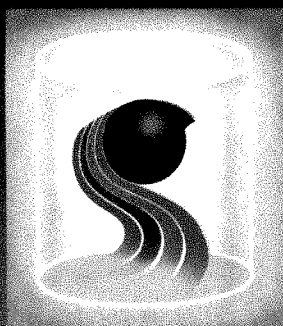
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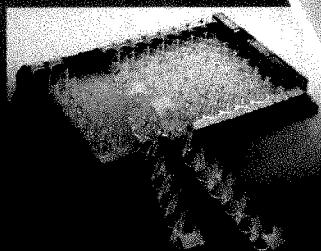
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## Gorbachev needs an answer

Mr Mikhail Gorbachev's visit to London last week has sharpened still further the issue of NATO's tactical nuclear weapons. NATO cannot pretend that the issue is technical and exclusively its own concern.

LAST year's tragic earthquake in Armenia has cast a long shadow. That is the simplest explanation why Mr Mikhail Gorbachev's visit to London last week was probably as much a disappointment for him as for many of those who heard what he had to say. His original plan had been to follow his speech at the United Nations in December, when he made public his plan unilaterally to cull 500,000 people from the Soviet armed services, by travelling to Cuba and then to London. Then, in the interregnum between the Reagan and Bush administrations in Washington, it would have been easier for him to open a fruitful dialogue on arms control. By last week, the momentum had been dissipated. Worse, the British government, now preoccupied with the sharpening dispute within the North Atlantic Treaty Organization (NATO) about the continuing role of nuclear weapons in Europe, was in no mood to respond imaginatively.

The issue is simple, but none the less corrosive on that account. The treaty on missiles of intermediate range (INF) signed at the end of 1987 regulates the removal from Europe of missiles capable of carrying nuclear warheads a distance of 1,500 km or more, but only implicitly regulates missiles of shorter range. (A precondition of INF was the readiness of NATO and the Warsaw Pact to remove tactical nuclear weapons from the scene.) Now, NATO wishes to improve ("modernize") the performance of its tactical nuclear weapons over the next few years. Specifically, there is a plan for replacing the Lance missiles now sited in West Germany with improved rockets capable of travelling 500 km. The Soviet Union disapproves — Gorbachev said so plainly last Friday. So, for different reasons, does West Germany.

Gorbachev's view is that improving the performance of NATO's tactical weapons will break the spirit if not the letter of INF. He said last week that if NATO goes ahead with its planned modernization, the talks on the regulation of conventional arms now under way in Vienna will be jeopardized. NATO's counter-argument is that nuclear weapons remain a necessary part of the defence of Western Europe. At least so long as the intended agreement on conventional forces in Europe remains in the future, nuclear weapons may be the only way of halting an armed incursion. That view is not new: NATO has held to the same doctrine for a quarter of a century. The novelty is twofold — there is now a chance of winning an agreement on conventional forces, while West German voters

and thus, perforce, the West German government are increasingly attracted by the even brighter prospect of a more general disappearance of political and military tension in Europe.

Formally, so far as NATO is concerned, the issue is intended to be settled in the summer of this year, after the Bush administration's review of its security interests (and the associated defence budget) is complete. But that is more than NATO's managers can reasonably expect. If anything, the reluctance of West German voters to support governments which agree to give house-room to modernized weapons is more likely to grow than to melt away. A further difficulty is that the Vienna negotiations will not make sense if aircraft and other remote means of delivering bombs (nuclear or otherwise) are excluded, which is what NATO asks.

That is why the need now is for a constructive compromise. Gorbachev's protest that NATO's insistence on improved nuclear weapons in Europe would jeopardize the Vienna negotiations was quickly countered, in London and Washington, by a simple reiteration of the need to modernize outdated nuclear weapons. Why not, instead, acknowledge that the need for battlefield nuclear weapons in Europe would be diminished if the Vienna talks succeed, and thus make the planned changes contingent on failure at Vienna? That way, Gorbachev and his counterparts in the West could be saying much the same thing, while the important negotiation at Vienna would be invested with a sense of urgency. □

## Disorderly publication

There is no reason why discoveries should not first be published in daily newspapers, but there are drawbacks.

THE great fuss about cold fusion over the past few weeks raises important questions about the scientific literature and its function to which the scientific community should give urgent thought. Reports that nuclear fusion had been brought about in an electrochemical cell first appeared in two financial newspapers — the *Wall Street Journal* and the *Financial Times* — on 23 March. Professors Martin Fleischmann (Southampton) and Stanley Pons (Utah) were reported to have accomplished fusion in an electrolytic cell.

Soon it also became known that a group at Brigham



Young University led by Professor Steven E. Jones was ready with comparable but not identical observations. The University of Utah promptly held a press conference at which, among other things, an article by Fleischmann and Pons was said to have been submitted to *Nature*. In the event, the first article to arrive was from the Brigham Young group, but one from Fleischmann and Pons reached us later; an extended version of it was published last week under the heading "preliminary note" in the *Journal of Electroanalytical Chemistry* (see page 537).

It is no disrespect to any of those concerned to compare the dilemma created for *Nature* by these events to that occasioned a year ago by the article in which Professor Jacques Benveniste and colleagues claimed that indefinitely diluted reagents retain their biological effectiveness. The claim flies in the face of orthodox belief, but the data available are insufficient for a careful judgement of its validity. But on this occasion, *Nature* has followed standard procedures. Both articles have been sent to referees, each is now being revised in the light of the many comments that have been made. Many of these would normally require extensive reinvestigation. In the normal course of events, weeks or months might go by. Researchers are used to delays of that kind, especially when they have important things to say. The process is beneficial because it improves the quality and reliability of what is published.

What is to happen now? Public and professional curiosity now require rapid publication, but there is only a small chance that either group of authors can make all the amendments asked of them in a short time. So should publication be postponed until they can do so? There is a sense in which that would not matter: the information is already in circulation. But publication is more than merely the circulation of information to those with access to the appropriate networks. General availability is crucial. That is why revised versions of one or other or of both articles will be published later in the month. In these exceptional circumstances, they will be accompanied by such comments of the referees as remain pertinent.

None of this implies that the peer-review system is infallible. Those who live with it know that it is shot through with imperfections. There is, for example, a danger that it induces too much uniformity in the literature. But the system is a powerful means by which good ideas are made better on their way into print. The practical question for the scientific community, and for journals in particular, is to adapt the system (and the process of publication) more swiftly to the steady improvement of communications.

Nobody can sensibly complain about those developments. But there remains an immense difference between the discussions thus stimulated within the scientific community and the broadcasting of claims not fully tested. Fleischmann and Pons said at their press conference that their work was in danger of "leaking out", much as Benveniste last year cited reports in *Le Monde* as a cause for urgency. But the authors of an experiment are best

placed to determine when and how news of their work is published generally. It is naturally difficult to bottle up exciting news, but impatience is a poor guide to action. The greater the importance of a discovery seems, the longer it should be worthwhile waiting to see it properly established. □

## Pan-European university?

The Swiss are nursing the idea of creating a European university and should be encouraged.

PLANS to turn the European Economic Community (EEC) into a genuine common market at the end of 1992 may not yet have done much to change economic behaviour in the 12 countries directly involved, but they have had a powerful influence on outsiders. Japanese and US companies are busy building factories in member states or forming business partnerships with companies registered there. Governments are similarly impelled by a sense of exclusion to seek membership of the EEC: Turkey has formally applied, Austria says it will and Norway (for the second time) is brooding. The way things are going, by 1992 there may be only one European outsider left outside — Switzerland.

This prospect keeps stoical Swiss awake at nights. Their difficulty is plain: the constitution of the Swiss Federation, with its devolution of power from the centre, could not be changed without changing Switzerland. Yet it was already plain last year (see *Nature* 336, 323 *et seq.*; 1988) that anxiety about the emerging single market had become an influence in Swiss planning of research as well as a source of fear that Switzerland may become an amalgam of holiday resort and retirement home for nationals who choose to spend their working lives elsewhere. But anxiety is a catalyst of the imagination, whence the notion now gaining ground that Switzerland, within the confines of its constitution, might make a distinctive contribution to the development of Europe by creating a university on a grand European scale.

This is a daring notion, but none the worse for that. Europe, taken as a whole, has a commendable diversity of universities, but most of them are bound to seem parochial by the yardsticks of the pan-Europeans. But Switzerland has a cosmopolitan tradition rivalled only by that of the Netherlands and might — so the calculation goes — be able to build from its existing institutions a distinctively European university that can be counted among the half dozen or so outstanding universities in the world. For the time being, of course, this is just a dream, but one which many influential Swiss are eager to explore. Altruism is not the only driving-force, of course: to be the site of an acknowledged leader among European universities would be an assurance against isolation, while a strong research programme would undoubtedly engender spin-off. But it is a notion that universities elsewhere in Europe should be ready to welcome. □

# Prospect of achieving cold fusion tantalizes

- Confirmation reports trickle in
- Dispute over primacy

## Washington

SCIENCE by press conference continued this week as Texas A & M University at College Station announced on Monday that researchers there had replicated at least one feature of experiments purporting to show nuclear fusion at room temperature. By the afternoon, a similar announcement had been put out by the press office at the Georgia Institute of Technology, Atlanta.

The Texas group, led by Charles Martin, is getting between 60 and 80 per cent more energy out of its experimental apparatus than it is putting in.

The Georgia group, led by Dr James Mahaffey, has measured neutrons from a palladium cell, recording a fifteenfold increase above background when current was flowing through the cell.

University of Utah chemist Stanley Pons, who first described the cold-fusion experiments at a press conference on 23 March, has confidently predicted that several groups would confirm his experiments. Other groups in the United States, working from preprints of a paper by Pons and Martin Fleischmann that has now appeared in the 10 March issue of the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* (261, No. 2a, 301-308; 1989), have not reported either the excess heat production or the appearance of 2.5 MeV neutrons.

Meanwhile, a legal battle is shaping up between the University of Utah and Brigham Young University (BYU) over who first discovered the cold-fusion process. The University of Utah filed a patent application during the week of 13 March, but officials at BYU believe that research done by faculty member Robert Jones along similar lines clearly precedes the work done by Pons. BYU will file a patent application shortly.

Paul Richards, director of public communications at BYU, says his institution felt no pressing need to obtain a patent, as applications for the technology were likely to be far in the future, and the results were of more scientific than commercial interest. Richards adds that BYU felt compelled to file a patent claim in response to accusations that Jones had stolen Pons's ideas.

The relationship between the BYU and University of Utah research teams has been rocky for some time. Last September, Jones was asked to review a grant application submitted to the Department of Energy (DoE) describing the Utah

team's efforts in cold fusion. The grant was ultimately approved. But as well as submitting his comments on the grant to DoE, Jones asked that DoE contact Pons and suggest a collaboration using a neutron detector Jones had developed. DoE did so, but efforts to bring the two groups together were unsuccessful. Ultimately the relationship between the two teams deteriorated to the point where the presidents of both universities met with the leaders of the two research teams to try to clear the air.

That meeting took place on 6 March. Both groups agreed to meet on 24 March at Salt Lake City airport to send simultaneous express packages to *Nature* containing their research results. The timing was crucial, as Jones had agreed to speak at a meeting of the American Physical Society in early May, and they were anxious to have a paper accepted by a peer-reviewed journal before then. Jones cancelled a seminar on his work that had been arranged for 11 March.

But in the intervening weeks, the University of Utah did two things that BYU considered in violation of the agreements worked out on 6 March. On 11 March, Pons and Fleischmann submitted a paper containing details of their cold-fusion experiments to the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, and on 23 March held a press conference to describe their findings. The BYU team was particularly annoyed when, during the press conference, University of Utah vice president for research, James Brophy, told reporters he was unaware of any other groups doing similar work.

The BYU group decided there was no longer any reason to delay, and sent its paper to *Nature* on 23 March. That at the University of Utah sent its on 24 March.

Brophy says the press conference was scheduled after the university learned that a local reporter was planning to publish a news item describing the findings. But Pons says the press conference was held because the paper had already been accepted by the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, although a news release from the journal's publisher, Elsevier Sequoia, states that the paper was accepted on 30 March.

Meanwhile, in Utah, the state legislature has set aside \$5 million to support fusion research once a state commission to be appointed by the governor is convinced that the findings have been confirmed. □

## SOVIET SUBMARINES

### Pollution fears for Norwegian Sea

#### London

A Soviet nuclear submarine of experimental design sank last week, with the loss of 42 lives, in international waters in the Norwegian Sea, between Bear Island and the Norwegian coast.

The immediate reaction in Norway has been one of consternation, even though Dr Willy Ostreng of the Fridtjof Nansen Institute (which studies security issues in northern waters) says that it "was only a matter of time" before such an accident happened in one of the world's most "heavily submarine-infested" channels.

Surface water and air samples collected by the Norwegian authorities have so far shown no signs of radioactive contamination. A Norwegian team is expected to reach the area on Thursday this week, with the intention of collecting deep-water samples, when it is hoped that a preliminary estimate of the immediate danger of contamination will be possible.

The submarine was equipped with two reactors, each of them cooled by a molten mix of lead and bismuth. If the fire which appears to have been the immediate cause of the disaster did not damage the integrity of the reactor compartments, and if the reactors were shut down before the ship sank, there is at least a chance that radioactive fuel elements will be entombed in a now-solid mass of metal from which radioactive material will be released only slowly.

As after the Chernobyl accident, the incident has become something of a test of *glasnost*. The Soviet side has released a certain amount of information, but *Pravda* complained at the weekend that Western journalists were able to get more information from Soviet embassies abroad than its own correspondents were able to gather in Moscow. Even so, the Norwegians are still clamouring for technical details so as to be able to calculate the long-term risk.

Vera Rich

## SOVIET UNION

### Reforms begin to take effect

#### London

THE Soviet Union announced last week that, under its new mental health legislation, more than 60,000 people have been taken off the registers of psychiatric clinics during the past year in Moscow alone. Eventually, it is expected that some 1.2 million Soviet citizens will be removed from the registers. How many names will remain on the registers is unclear. But the new legislation stresses that no citizen may now be hospitalized without his or her consent, except for people "posing an immediate threat to society".

Vera Rich

## Culprit found at Cornell

### Washington

BLAME for a computer 'worm' that crippled thousands of installations on the Internet computer network last November has been placed squarely on the shoulders of Robert Tappan Morris by a commission of inquiry at Cornell University, where Morris is a graduate student.

The attack raised many questions about the security of computer networks to unwarranted entry. But according to the Cornell inquiry, made public late last month, Morris's actions did not so much represent "an heroic event that pointed up the weaknesses of operating systems" as "a juvenile act that ignored the clear potential consequences".

The worm was released on 3 November, 1989 and infected computers using particular versions of the UNIX operating system (see *Nature* 336, 97; 1988). Although it was first diagnosed as a computer 'virus', the infecting program is more properly known as a worm, as it does not require another computer program to act as a 'host' to carry it from one machine

to another.

According to the inquiry's findings, the worm consisted of two parts; a 99-line 'probe' written in the 'C' programming language, and a much larger 'corpus' that had been compiled into machine language. If the probe was able to get into a computer, it sent for its larger body. The probe used four basic methods to gain entry. Two used specific design features of UNIX mail programs that allowed the worm to enter a new machine without passwords that would normally be required. Another avenue was essentially a sophisticated guessing game in which the worm exploited a human tendency to choose simple passwords related to account names. A fourth method exploited a design feature of UNIX that allows people to use the same password on several different machines where they have accounts.

After releasing the worm on the evening of 3 November, Morris evidently decided he had made a mistake, and asked an associate at Harvard to send a message to computer users around the country telling them how to protect themselves against the worm. But the warning was sent by a slow method, and arrived after others had discovered the worm and developed ways to defeat it.

The commission concluded that several thousand computers were infected by the worm, but thousands more had to be shut down as a precaution to prove that infection had not occurred.

The commission points out that Morris was able to exploit security flaws in the UNIX operating system but that UNIX was never designed to be a secure system and many of the flaws were already well known. The commission concluded that creating the worm required "dedication and perseverance rather than technical brilliance", adding that Morris "displayed naive conceit in assuming that he could launch an untested, unsimulated, complex worm onto a complex network and have it work correctly the first time".

Morris declined to be interviewed by the commission of inquiry on the advice of his counsel, but from talking with his colleagues and friends and by examining logs of Morris's computer activities, the commission concluded that Morris alone had written the worm.

Although the commission recommends strong disciplinary action, it suggests that the discipline "should not be so stern as to damage permanently the perpetrator's career". Cornell University has not yet decided on any punishment for Morris. An investigation of the incident by the Federal Bureau of Investigation has not yet resulted in any indictments.

Joseph Palca

## Britain to expand?

### London

BRITAIN has played only a minor role in Arctic research until now, with total funds from the research councils of little more than £1.5 million last year. The Natural Environmental Research Council (NERC) now wants to change this, so that Britain can act at least as a useful partner in international research programmes.

Last year, NERC spent less than £500,000 on Arctic research, mostly on individual research grants and training awards; there are no institutional research programmes devoted to the Arctic. NERC has now asked for an additional £2 million a year from next year's science budget for Arctic research.

The council has rejected the suggestion that it create an Arctic research institution with permanent research bases comparable in size to those in the Antarctic. "On political and financial grounds", this would be unrealistic, it says in its first strategy document for the Arctic, published this week. Less ambitiously, it wants to build a small base on Svalbard, the archipelago north of Norway. The base would be intended to house about 20 researchers and to cost only £70,000 to build and £50,000 a year to run.

The council also wants to increase support for the Scott Polar Research Institute at the University of Cambridge. With more staff and increased and secure long-term funds, this institute would become the focus of British Arctic research, says Dr John Bowman, secretary of the council. But he stresses that expansion here will not compromise research elsewhere in the country. Research areas to which the council gives priority are meteorology, solar-terrestrial physics, oceanography, glaciology, ecology, geology, socio-economic sciences and engineering.

Christine McGour

## Explorers selected

### Washington

The National Aeronautics and Space Administration (NASA) last week announced the first four in a series of small scientific missions that will be launched on existing expendable launch vehicles. The small scale missions — with total costs for each spacecraft expected to average \$30 million — are intended to provide research opportunities for a new generation of space scientists and engineers.

The four projects, chosen out of 51 applications, include the Solar, Anomalous and Magnetospheric Particle Explorer, the Submillimeter Wave Astronomy Satellite, the Fast Auroral Snapshot Explorer and a global ozone satellite that will carry a total ozone mapping spectrometer.

Joseph Palca

## Pakistani virus invades Indian neighbour

### New Delhi

'C-BRAN', a computer virus of Pakistani origin, has hit personal computers throughout India in what is thought to be the first epidemic of its kind in the nation. The incident highlights India's extensive trade in pirated software and copied floppy disks.

In just four weeks, 'C-bran' showed up in computer schools and a private company in Bangalore and in some 20 companies in Bombay, including an international bank. The virus was also reported from Madras, Kanpur and Vijayawada, a small town where 750 of 1,000 floppy disks used by a private company were found to be infected. Scientists at the Delhi University Computer Centre said their machines were invaded by the 'ashar' variant of the virus.

Dr N. C. Kalra of the Indian Institute of Technology in Delhi, who detected 'ashar' in the institute's computer, centre blamed the epidemic on pirated software. No virus has yet shown up in computers linked by electronic networks but government agencies have been alerted. India's NICNET links the Cybercomputer main-frame computer in the Planning Commission with portable computers in district headquarters while another network, ERNET links the computer centres of eight educational institutions. The six major commercial computer networks all claim to have strengthened their security system following the epidemic. K.S. Jayaraman



## RAIN FORESTS

# Disputes about destruction

## São Paulo

By quoting figures that downplay the extent of Amazon deforestation, Brazil's President José Sarney last week touched off a dispute over estimates of rain-forest destruction and embarrassed his nation's own Instituto de Pesquisas Espaciais (INPE, Institute of Space Research).

Sarney's comments came as he signed into law legislation that creates the 'Nossa Natureza' (Our Nature) programme designed to protect the Amazon forests (see *Nature* 338, 286; 23 March 1989).

He harshly attacked 'foreign intervention' in Brazil's Amazon policy and, to prove that the destruction of Amazon rain forest is not as bad as foreigners claim, he noted that the latest data on deforestation calculated by INPE scientists show that just 5.12 per cent of the rain forest has been cleared since the colonization of Brazil began in 1500.

But that figure is in dispute. A 'green' federal deputy, social-democrat Fábio Feldmann from São Paulo, claimed the data had been 'manipulated' to suit government needs. The '5.12 per cent' derives from figures on the destruction of tropical moist forest. But the INPE team responsible for the analysis related the area destroyed (250,429 square kilometres) to the total area of the Brazilian Amazon region,

rather than to the total area of rain forest, which is smaller (see map).

Barbosa said the team did not relate deforestation data to total rain-forest area because "we do not know the true extent of the rain forest". But other INPE researchers said they thought it was a mistake to present the data that way. To pre-



The Brazilian Amazon is legally defined as consisting of the whole of six states (Acre (AC), Amazonas (AM), Rondônia (RO), Roraima (RR), Pará (PA) and Amapá (AP) and parts of three others (Mato Grosso (MT), Maranhão (MA) and Tocantins (TO)). Only about three quarters of its 4,906,784 square kilometres contains rain forest. The remainder consists of savannah.

pare the report for the president, a task force was rapidly assembled and a building made over to it. In a vain attempt to prevent leaks to the press, INPE even moved its press officers away from the remote-sensing team.

Recalculation of the INPE figures using an estimate of the total area of rain forest (3,700,000 square kilometres) suggests that 6.8 per cent of the rain forest has been destroyed. But that figure is still much smaller than the 12 per cent deforestation claimed by the World Bank in its report *Government Policies and Deforestation in Brazil's Amazon Region*, released in January.

That figure is based largely on projections from Landsat data published in 1986 by Philip Fearnside, a US scientist working at the National Institute of Amazon Research at Manaus. But Fearnside himself estimates Amazon deforestation at 8 per cent. INPE's director-general, Márcio Noqueira Barbosa, says his institute's estimate is more accurate because it is based on 1988 Landsat images.

Landsat images are received every day at INP's station in Cuiabá, Mato Grosso. INP remote-sensing director Roberto Pereira da Cunha's team used Landsat Thematic Mapper data, with 30-metre resolution in the visible and near-infrared regions, to compile its data on deforestation. Cunha, who is clearly shaken by the controversy caused by the data, says they have studied 234 images, of which 104 were selected for detailed interpretation because they contained deforested regions. He claims the research began in August 1988 but this too is disputed. Other scientists say work began in earnest only in late March this year.

Ricardo Bonalume Neto

## SSC

## India offers help

### New Delhi

INDIA is proposing to participate in the US Superconducting Super Collider (SSC) project by contributing manpower and equipment but not money, according to Science Minister K. R. Narayanan. He told parliament last week that informal discussions had taken place between Indian and US scientists but "no formal agreement has been signed nor any commitment on expenditure agreed to".

Narayanan said India was joining the SSC project because it provided "a good opportunity for Indian scientists to work at the frontiers of science and technology".

A spokesman for the Indian team negotiating with the SSC project authorities confirmed an Indian offer of \$50 million in hardware, particularly particle detectors, but "there is not going to be any monetary contribution at all", he said.

K.S. Jayaraman

## CLIMATE

## Worldwide atmosphere authority resisted

### Paris

AN official advertisement in French newspapers setting out the declaration signed by 24 nations at last month's environment conference at the Hague (see *Nature* 338, 193; 1989) has provoked an angry reaction in Brazil. The advertisement appeared on Monday, 3 April in all 24 signatory countries, but the version in *Le Monde*, *Figaro* and *Libération*, published at a cost of FF1.8 million (\$290,000) to the prime minister's information service, reproduced the signatures of the 24 national representatives alongside an additional statement which, it seems, was not part of the declaration.

The statement says that the 24 signatories call for the creation of "a worldwide authority, invested with real decision-making and executive powers to save the atmosphere". The statement also says that the signatories are "ready to surrender part of their national sovereignty for the common good of humanity as a whole".

Paulo Tarso Flecha de Lima, the Brazilian diplomat whose signature appears in the advertisement, was not prepared to go this far at the Hague. The Brazilian government, criticized for destruction of its rain forests, has angrily resisted attempts by other nations to enforce a policy of conser-

vation. Brazilian President José Sarney recently accused the foreign media of conducting an "alarmist campaign" against Brazil and complained that developed countries were backing policies that would make Brazilians "slaves".

The United States, which did not send a representative to the Hague meeting, also has no enthusiasm for a new worldwide authority. At a congressional briefing last week, William A. Nitze, a US State Department representative, said it was "premature to be considering a 'law of the air' or supranational authorities to deal with climate change".

With European elections in full swing in France, concern for the environment has become a campaign issue. Opinion polls following a good result in recent municipal elections have shown the hitherto dormant French ecology party to be an emerging alternative centre force and both the incumbent socialists and the right-wing opposition have been scrambling to demonstrate their interest in environmental issues. That may partly explain the government's decision to advertise the Hague declaration with President François Mitterrand's signature prominently on display.

Peter Coles

## New sales tax hits science

### Tokyo

JAPAN'S controversial new sales tax, which came into effect last week, has not only placed an additional burden on Japan's consumers but has also pushed up the costs of scientific research. The budgets for the science-related ministries have been increased over the past few months to cover some of the costs of the new tax, but universities and research institutes will still find themselves worse off.

The sales tax marks the most sweeping reform of Japan's tax system since the Second World War and has met almost universal opposition. Although set at a low overall rate of 3 per cent, the tax covers almost all forms of 'consumption'. Books, food, transportation, rent and even the costs of childbirth and tombstones are taxed. Scientific research is no exception.

Although the tax takes up only a small percentage of ministerial budgets, it does constitute a significant proportion of the increase in outlay for science between the fiscal years 1988 and 1989 — about 20 per cent, or ¥5,900 million (\$45 million), in the case of the Science and Technology Agency.

The tax is levied on the purchase of equipment, books and journals and also adds significantly to the cost of construction of new facilities. Although the various science-related ministries have made upward revisions in their budgets to cover the tax, some of the burden will inevitably have to be borne by the universities and research institutes.

For example, although the Ministry of Education, Science and Culture has raised the budget for fiscal year 1989 for grants-in-aid of research to cover the increased costs of equipment, the value of multi-year grants awarded before implementation of the tax (for example, the 'special distinguished grants' which run for 3–5 years) will remain unchanged.

The new tax has helped to make Noboru Takeshita the most unpopular prime minister in the post-war era. The latest polls show that less than 10 per cent of the public support his administration. And revelations last week that Takeshita received almost ¥100 million (\$760,000) in donations from the scandal-ridden Recruit company in the run-up to his election as head of the ruling Liberal Democratic Party (LDP) have further weakened his position.

Calls for Takeshita's resignation are growing within the LDP and he may not be able to survive in office for longer than a few more months. Under a new leader, the sales tax could be revised, but as it has taken the LDP ten years and two failed attempts to introduce it, the tax seems likely to remain.

David Swinbanks

## More yen for joint research

### Tokyo

LEGISLATION at present before Japan's Diet will allow the Science and Technology Agency to launch a new international programme in October to promote joint research with overseas institutions. The new programme will for the first time open a way for Japanese government money to be spent on research facilities in foreign countries.

The new research initiative will be modelled along the lines of the agency's Exploratory Research for Advanced Technology (ERATO) programme, which recently received high marks in an assessment by US researchers (see *Nature* 337, 196; 1989). ERATO, which is run by the agency's Research and Development Corporation of Japan (JRDC), finances 'high-risk' research with potential for application. Although some foreign scientists participate in ERATO, the research is carried out entirely within Japan. The new 'international ERATO' will allow participation by overseas research organizations (in government, industry and university) through cooperative research agreements. Legislation allowing JRDC to establish such agreements is expected to pass through the Diet within the next month or two, say agency officials.

The programme will support teams of about 20 researchers, about half based in Japan and half overseas, with funds of about \$8 to \$13 million for periods of 3 to 5 years. Agency officials hope that participating countries will contribute research funds and facilities as well as researchers, although it is possible that JRDC will 'rent' research laboratories overseas as the corporation now does in Japan for ERATO.

Until now, it has been almost impossible for Japanese government organizations to spend money on research facilities in foreign countries because of the lack of rules and mechanisms for auditing and accounting. A classic example is the bureaucratic delay encountered by Tokyo Astronomical Observatory in its attempts to build the world's largest telescope in Hawaii (see *Nature* 311, 5; 1984). After years of debate, the telescope has still to win government approval. By renting rather than buying research facilities, agency officials are confident they can avoid such problems.

Masahiko Noda of the agency's International Affairs Division says the programme received a favourable response in the United States last month when he visited various organizations, including the Office of Science and Technology Policy, the National Science Foundation, universities and non-profit research institutions. And Kaname Ikeda, director of the division, says a research project with the

United States could be set up within a few months using the new mechanisms created under the US-Japan Science and Technology Agreement signed last June.

But some difficulties may be encountered in trying to apply the ERATO model to the United States. ERATO projects are headed by senior Japanese scientists hand-picked by JRDC, an approach that runs counter to the open and competitive research system of the United States. And agency officials admit that they will probably have to adopt a more "flexible" approach in foreign countries than that used in Japan.

Apart from international joint research projects, the new organization in JRDC will administer the agency's new post-doctoral fellowship scheme for foreign researchers which began last year (see *Nature* 335, 287; 1988), and will provide accommodation, Japanese language training, counselling and information for overseas researchers working in Japan. The agency has set aside ¥418 million (\$3.2 million) for the programme in the second half of fiscal year 1989 and about ¥800 million to build 50 homes for foreign researchers in Tsukuba Science City by 1991.

David Swinbanks

### HIGHER EDUCATION

## Vet schools reprieved in Britain

### London

FEARS that two of Britain's six veterinary schools may have to close receded last week when the Universities Funding Council decided to ask the Ministry of Agriculture, Fisheries and Food (MAFF) to reconsider future manpower requirements in the veterinary profession. A review is expected to show that previous forecasts of manpower needs were too low and that all six schools are necessary.

A report published in January recommended the closure of the veterinary schools at the Universities of Glasgow and Cambridge and the redistribution of their staff to the remaining four schools (see *Nature* 338, 191, 16 March 1989). But the British Veterinary Association now argues that more students are needed and that earlier estimates it gave to MAFF were wrong.

The decision was welcomed by James Armour, head of the veterinary school at the University of Glasgow. But he remains dissatisfied that the recommendation to close Glasgow was partly based on its proximity to Edinburgh, where there is another veterinary school, rather than solely on academic performance.

Christine McGourty

## ALASKA OIL SPILL

# Fisheries first to suffer

## Berkeley

A HUGE clean-up operation continued last week in Alaska's Prince William Sound, the site of the largest oil-tanker spill in US history. As the main oil slick moved southwest, out of the sound, it left behind smaller slicks, hundreds of miles of contaminated shoreline and more than 1,400 square miles of fouled waters. The wrecked tanker *Exxon Valdez* was floated from the reef where it had run aground and towed to a nearby island for repairs.

The captain of the tanker, Joseph Hazelwood, is said to have been under the influence of alcohol and absent from the bridge at the time of the accident. Coast Guard investigators were told by at least one crew member that Hazelwood had placed the ship on automatic pilot on an erroneous collision course with the reef.

Hazelwood, wanted in Alaska on misdemeanor charges associated with the spill, surrendered to the authorities in his home state of New York last week. A New York judge set his bail at \$1 million, after pointing out the magnitude of the disaster, but a second judge later reduced bail to \$25,000.

The oil contaminated a large portion of the herring-spawning grounds in Prince William Sound. Last week, Alaska Fish and Game officials cancelled the herring-roe fishery that would normally have opened several weeks ago. Paul Ruesch, a state fisheries biologist, said both herring eggs and young larvae are highly susceptible to toxic hydrocarbons. "We couldn't justify additional mortality from the fishery", he said. There is also a risk that the roe may be contaminated and therefore unsaleable. The fishermen, who stand to lose \$12 million, hope to recover damages from Exxon.

This year's \$70-million salmon fishery, due to begin in early summer, may escape serious damage from the spill, but the catch in future years is likely to be reduced if the oil affects this year's young. Fishermen continued to set floating booms to keep oil out of the bays that house the area's four salmon hatcheries.

But Ruesch acknowledged that success with booms has been "spotty", and the hatcheries are largely at the mercy of the winds. If large amounts of oil come their way, salmon fry reared in captivity may be caught at a vulnerable stage when they are being transferred to salt-water pens ready for their May release. Wild fry which are beginning to emerge from gravel beds will also be at risk.

The level of toxins in the sound, and their effect on the plankton, is not yet known, as water sampling has just begun. Various agencies are preparing for long-term studies of the effects of the oil on water quality, sediments and beaches.

"There will undoubtedly be some very long-term persistent impacts", said Ruesch. "Prudhoe Bay crude does not weather well; it doesn't break up and it doesn't sink. It is very persistent."

The sound has the largest concentration of sea otters in the world, according to Mark Kuwada, a habitat biologist with the fish and game department. Kuwada said that 30 per cent of the sound's 5,000 sea otters live in areas affected by the spill, although it is not known how many have died.

Thousands of waterfowl have been killed by the oil, and the sound's large population of endangered bald eagles has been reported to be feeding on the contaminated carcasses, although no dead eagles have yet been found. Two deer were found dead after apparently feeding on seaweed tainted with oil.

President Bush last week made available US military troops to help clean up the spill, acknowledging that Exxon's efforts were insufficient. He emphasized that the government was not "federalizing" the clean-up operation, and that Exxon would still be responsible for costs.

Environmentalists say an accident such as this was inevitable, given Alaska's laxity in monitoring the oil industry, which supports 85 per cent of the state's economy. Mike Matz, of the Sierra Club, said Alaska's Department of Environmental Conservation, which is responsible for approving the oil industry's contingency plan for clean-up of spills and for assuring that the industry maintains the promised level of preparedness, lacks adequate funds and has been unable to carry out adequate monitoring. Even when permit violations were discovered, said Matz, political pressures prevented prosecution of the violators.

Although the spill has not changed the Bush administration's support for oil exploration in Alaska's Arctic National Wildlife Refuge (ANWR), public outrage over the accident has slowed the progress through Congress of a bill to authorize the exploration, while both houses hold hearings on the causes and potential effects of the accident. Congressional representatives from California have used the spill to fuel their protest against the opening of areas off the California to oil exploration. Interior secretary Manuel Lujan Jr warned oil industry representatives last week that the *Valdez* accident could have an effect on the oil industry similar to that of the Three Mile Island accident on nuclear power. "If the image of an uncaring and uncaring industry prevails among the public", he said, "then we can kiss goodbye to domestic oil and gas development in ANWR, offshore, and in the public lands."

**Marcia Barinaga**

## FRENCH RESEARCH

# Worries about future

## Paris

THE French research minister, Hubert Curien, has set up two working parties with unions representing researchers to find ways to protect the future of their profession. One will consider the problem of recruitment of young researchers to the major state research organizations, such as INSERM and CNRS, while the other will look at career prospects, with the accent on greater mobility of trained researchers towards universities and industry.

The unions are pessimistic about the negotiations. Robert Descimon, general secretary of SNCS, the biggest union representing full-time researchers, fears that the government is dismantling the state research organizations in favour of the universities, whose research role in many disciplines is relatively small. Most research is carried out in state organizations, where researchers have jobs for life and a small teaching load. But a slowing in the number of new posts has created a bottleneck of young postdoctoral researchers seeking their first job, with a resulting rise in the average age of researchers. Meanwhile, salaries are becoming increasingly unattractive compared with those offered by industry, even to untrained researchers.

In his pre-election "letter to the French people", President François Mitterrand declared that research would be a national priority. "There is not much evidence of this so far", says Descimon. Most of the government effort, he says, is directed towards major reforms of education, including higher education. The unions fear that trained researchers will be drained from the state research organizations in order to inject new blood into the universities, without the promised reevaluation of research as a career.

Peter Coles

## JAPAN

# Electronic Leonardo

## Tokyo

JAPANESE art lovers who want to see the Mona Lisa but cannot make the trip to the Louvre may soon be able to enjoy the next best thing. A new art gallery in Gifu, central Japan, is using high-definition television (HDTV) to display works of art recorded digitally on compact discs.

HDTV, which was first developed by Japan's National Broadcasting Corporation (NHK), has twice as many lines on the screen as conventional television and produces images of exceptional clarity.

The Gifu Museum of Fine Arts is using the system to show pictures from its collection which it does not have room to put on display. In the future, museum officials hope to import HDTV images from art galleries all over the world so that visitors will be able to view famous works of art at the push of a button.

David Swinbanks



## Activists attack in Arizona

### Tucson

ANIMAL rights activists broke into four buildings at the University of Arizona on 3 April and set two fires, vandalized laboratories and kidnapped 1,000 animals to protest against the use of animals in research. University officials said that structural damage amounted to \$110,000 and estimates of the total damage go as high as \$250,000.

In press releases left at local newspaper offices and radio and television stations, the Animal Liberation Front claimed responsibility for the arson and damage. A fire in a rooftop laboratory of a five-storey pharmacy-microbiology building gutted two rooms and damaged two others, causing a total of \$90,000 damage. That fire took an hour and 46 firefighters to get under control.

A second fire was started in the crawl space under the small house containing the animal resources administrative offices and caused \$10,000 damage. Clyde S. Card, director of the Arizona Diagnostic Laboratory, said that about \$125,000 damage had been done to that laboratory's autopsy room and three veterinary science laboratories.

Vandals also smashed windows and ransacked laboratories in a neighbouring building and in another a block north of the pharmacy-microbiology building. They spray-painted slogans including "Nowhere is safe", "We shall return", "You are killers" and "Torture teaches nothing" in red on the walls and floors. Pet food was strewn over one basement.

The group's statement said that a thousand animals were liberated as an act of mercy and compassion for the individual animal victims and that laboratories were damaged to protest against vivisection. The statement claimed that the action was part of an international campaign against misguided efforts by the scientific and medical industry.

'People for the Ethical Treatment of Animals', an animal rights organisation based in Washington, DC, later provided television stations with a video of the break-in, showing black-hooded individuals loading animals into boxes and smashing windows with hammers. The group took about 1,000 mice, 50 rats, 175 rabbits, 4 frogs and several guinea pigs. According to the animal activists, the animals were being placed "in good homes around the country where they will live free from the invasive curiosity of researchers and vivisectionists".

About 30 of 340 mice taken from one building were infected with *Cryptosporidium*, a protozoa that causes severe diarrhoea, according to Charles Sterling, a professor of veterinary science.

The mice were being used in a study

supported by the US Environmental Protection Agency to test the effectiveness of disinfectants against this waterborne parasite. Those who handle the mice may suffer from severe diarrhoea for two to four weeks.

Other affected work included genetic studies of heat stress, studies of the long-term effects of selenium-deficient diets, research on a vaccine for swine dysentery and studies of the effect of diet on protein metabolism. Scientists involved estimated that the loss of animals set the projects back months, and perhaps years.

The Animal Liberation Front group may have been helped by people inside the university as they appear to have known where and how the animals were housed, according to Michael Cusano-vich, University of Arizona vice-president for research. The group is thought to have broken into one building by forcing open a vent. Police were not sure how the group gained access to the other buildings. The Federal Bureau of Investigation is also involved in the case.

In January, animal activists claimed responsibility for taking seven dogs used in heart research at the Tucson Veterans Administration Hospital.

**Elizabeth Pennisi**

## Municipal law on its way

### Boston

WITH recommendations from an advisory panel due this week, the city of Cambridge, Massachusetts, moves one step closer to passing the first local ordinance in the United States to provide municipal control over the care and treatment of laboratory animals. While it is still too early to know precisely what provisions the law will entail, it is likely that the city council will pass legislation this spring.

The new recommendations come from a panel established by the city council in the autumn of 1987 to evaluate the treatment of laboratory animals at facilities in Cambridge. Among those facilities are several belonging to the Massachusetts Institute of Technology (MIT).

The three-member panel, which includes a local veterinarian and representatives from the scientific community and animal rights groups, found no instances of cruelty or abuse of animals in Cambridge laboratories. But the group is still split over the adequacy of existing regulatory mechanisms in the city.

Because of the disagreement, the panel delayed release of its recommendations to the city council. But the pressure is on for city officials to act on the issue. Under consideration are provisions which would give a local animal commission the power to uphold "the community's ethical standards for the use of animals in experiments". The local authority would investigate alleged violations of local standards for the care of laboratory animals and fines to research institutions that failed to comply. A further provision to be considered by the city council would require researchers who handle animals in laboratories in Cambridge to take a course from the local animal commission on the new standards.

John Moses, an MIT physician who heads the university's animal care committee, represented the scientific community on the advisory panel. He stresses

that the panel's lack of evidence of animal abuse argues against the need for local legislation. But he believes the panel is "very close" to making recommendations that all three representatives can agree upon. The recommendations should give a clear guide as to the severity of the city council's actions.

The council has already taken interim action. Nearly two years ago, when the advisory panel was formed, it banned outright within city limits the use of two common animal toxicity tests, the Draize test and the LD 50 test. Animal rights groups have already proposed strong city legislation to regulate practices involving laboratory animals; the panel's forthcoming recommendations are seen as offering the potential of a politically viable compromise.

With the week of 24 April designated by animal rights activists as 'world week for lab animals', increased public attention is expected. In Massachusetts, organizers say they plan to hold demonstrations aimed specifically at the Gillette Corporation because of the company's use of animals in the development and testing of its products.

In New York, for the third year in a row, protesters plan to target their demonstrations at New York University. Animal rights groups have singled out the research conducted by Ronald W. Wood, professor in the department of environmental medicine. Wood, who studies the effects of the inhalation of toxic substances including crack and cocaine, as well as organic solvents, uses rodents and some primates in his research. Last year's demonstrations in New York drew more than a thousand protesters.

Wood says he believes most are "well intentioned, but misguided". "My work", he says, "tries to provide information about very real human problems of drug abuse and occupational hazards."

**Seth Shulman**

## Different ways with *glasnost*

London

Mr Mikhail Gorbachev, the Soviet president and Party general-secretary successfully tied up London traffic for two days last week, made a speech at the Guildhall, had lunch with the British Queen, who accepted his invitation to visit the Soviet Union at a "convenient" time.

The official visit to London followed one to Cuba, both of which had been postponed in December on account of the Armenian earthquake. But the changed itinerary allowed Gorbachev to meet the Prime Minister of the Irish Republic during a stopover on the outward journey.

The three visits showed some interesting contrasts in the exercise of *glasnost*. In Ireland, Gorbachev stressed his concept of a "common European home" in language apparently innocent of Ireland's jealously guarded neutrality, but agreed with Mr Charles Haughey on further economic collaboration in fishing, shipbuilding and barter in food products.

Gorbachev promised to raise in London two human-rights issues arising from the continuing conflict in Ulster (Northern Ireland), but declined to suggest a political solution for the conflict.

In Cuba, where President Fidel Castro has so far proved remarkably resistant to *perestroika*, the emphasis was on continuing friendship. The two leaders signed a new treaty of cooperation in science and

technology, and promised improved contacts in "science, culture, education, health-care, the press, radio, television, cinema, tourism and sport".

But Gorbachev gave a clear warning against the "export of revolution" from Cuba to Latin America and visited a science exhibition showing a new high-powered sugar-cane harvester, a micro-analysis system for blood testing and a model of the Juragua nuclear power station being built with Soviet assistance.

In Britain, Gorbachev used his Guildhall speech to announce that the Soviet Union has ceased the production of weapons-grade plutonium, and that it is about to do the same for weapons-grade uranium. This was described by the US State Department as an "empty gesture" on the grounds that the recycling of existing stocks of fissile material should suffice for many years to come. Mrs Thatcher, the British Prime Minister, rather emphasized her view that nuclear weapons provide a stronger peace-keeping deterrent than conventional forces.

On the human rights front, the Gorbachev visit was marked by the arrival in London of mathematician Georgii Samoilovich, long denied an exit visa on the grounds of his access to classified information 17 years ago. He is to undergo urgent treatment for a leukaemic condition.

Although the Soviet and British governments agreed during the visit on further cooperation in the arts and trade, little was said at the formal level about science and technology. The Soviet offer to find a place on a future space mission for a British astronaut, now made unconditionally, seems to have run into British unwillingness to meet the costs entailed.

Professor Vitali Goldanskii, director of the Institute of General Physics in Moscow, well-known in Britain as the chairman of the Soviet Pugwash committee, was prominent among Mr Gorbachev's advisers during the whole week. As one of the first to recognize that some radioactive nuclei may emit particles heavier than  $\alpha$ -particles, he was one of those who visited the Atomic Energy Research Establishment to inspect the still-negative attempts to replicate electrolytic fusion. He was also eager to explain to all enquirers how he proposes, as part of *perestroika*, to split his Moscow institute into several autonomous units, each with a separate director.

Gorbachev himself visited a British factory manufacturing communications equipment supplied to the Soviet Union — and *Izvestia* took the occasion to lecture the West on the iniquities of the Cocom ban on the export of sensitive technology to the Socialist bloc.

Vera Rich

## Remote-sensing satellite for Iraq

São Paulo

Brazil's Institute of Space Research (INPE) is considering a proposal to build a remote-sensing satellite with military potential for Iraq. Brazil was an important supplier of arms to Iraq during the Iran-Iraq war.

INPE, together with Brazil's aircraft manufacturer, Embraer, and private companies would build the body of the satellite using technology INPE has acquired for its own programme to construct four satellites. Brazil will have its first satellite ready by the end of the year, although it may have to wait until 1992 for an indigenous launch. The development of the Satellite Launch Vehicle by the Air Force is behind schedule.

The first two Brazilian satellites will be small (115 kg) test capsules. Remote-sensing satellites of similar size will then be launched. INPE also has an agreement with China to design a bigger remote-sensing device.

Iraq is thought to be interested in help in establishing a satellite-building and testing facility. The sophisticated camera required for military sensing would come from a French company. But the deal appears to be encountering opposition inside Brazil's Foreign Ministry. INPE's director-general, Marcio Nogueira Barbosa, said he would prefer to pass INPE's satellite-construction technology to private companies.

Ricardo Bonalume Neto

## Study planned of greenhouse effect

Sydney

A YEAR after rejecting a request for A\$1 million to support research into the greenhouse effect, the Australian government has changed its mind and agreed to spend A\$7.8 million on the same topic over the next 15 months.

The Prime Minister, Bob Hawke, explained the turn-around by stressing the "dramatic explosion of awareness" of the greenhouse effect over the last year.

Part of the A\$7.8 million will be used to set up a National Greenhouse Advisory Committee of six experts to help the government to set research priorities. A\$5.54 million will go to the Commonwealth Scientific and Industrial Research Organization (CSIRO) and the Bureau of Meteorology to continue research into predictive modelling. The remainder will support Australian participation in international research programmes.

The government has also promised to help countries in the Pacific region that will be affected by changes in sea level.

Tania Ewing

### NEWS IN BRIEF

## New radiotelescope

Bangalore

Under a new agreement, India and the Soviet Union will jointly fabricate and install a radiotelescope in South India.

The telescope will form part of an eastern hemisphere very-long-baseline interferometer. It will be linked to the baseline connecting similar radiotelescopes at Ussurisk and Ephratoria in the Soviet Union.

R. R.

## Prisoners freed

Washington

TWELVE Somali scientists, engineers and medical doctors were freed from prison earlier this month, according to two committees of the US National Academy of Sciences and the Institute of Medicine which had taken up their cause. A report published by the two committees last year accused the Somali government of torturing prisoners and of detaining them for years without formal charges (see *Nature* 331, 196; 1988). Somali Prime Minister Lt General Mohamed Ali Samantar promised in a speech in Washington last month that his government would release the prisoners.

J. P.

## Having the final word. . .

SIR—I do not know whether there are still Creationists who believe that God created the world with implanted fossils and strata records (and similarly a Universe with implanted galactic records); but, if there are, then Bruce Denness's letter "Divine artefact" (*Nature* 336, 614; 1988), raises a problem that should be of great concern to them.

"God is not a man, that He should lie" (Numbers 23:19). Yet the suggestion that God could have created a world, or Universe, in which a record of the past was 'implanted', implies that he has lied. And the consequences to faith could be more shattering than they are to science.

First: if (*arguendo*) the Earth and Universe are (say) only 20,000-odd years old, but have been created to appear respectively about  $5 \times 10^9$  and  $20 \times 10^9$  years old, then these greater ages are without doubt their *scientific* ages — there is no way that science can come up with an age of 20,000 years. To understand this, consider what the *scientific* ages of brand-new but exact copies of this Earth and Universe would be, if God chose to make such copies.

Second, it becomes a matter of faith alone, under such circumstances, that the age of the Earth or Universe is anything other than what it appears to be scientifically. And only faith can govern the selected age. But why, under such circumstances, should any particular age be arbitrarily chosen? Creationists may interpret the Bible as implying one age rather than another; but the Earth could have been created *some year ad* with Bibles intact as part of the implanted record. How is one to prove that that did not occur? But if it did, then Jesus was not born on Earth and did not die for us; nor was he resurrected. What happens, then, to our salvation? So, to believe that God could have created an Earth or Universe with an implanted record ultimately strikes at the heart of the Christian faith. Maybe quite a headache for Creationists.

Perhaps there is no scientific answer to the problem Denness raises, but only a theological one.

JOHN BLASDALE

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SIR—I find that it has become necessary, for a second time (see *Nature* 323, 754; 1986), for Me to intervene in order to present My Word in these pages.

Unless I have misunderstood matters (which is unlikely), I am depicted by some individuals as a sort of Divine Con-Artist (*Nature* 336, 614; 1988 and 337, 498; 1989), allegedly creating the Universe, and then disguising this fact, for reasons apparently known only to Myself. Others find it beholden upon themselves to rush

to My defence and deny this, because they have decided that it reflects poorly on My Divine Logic.

I had hoped that My previous Correspondence in these columns had made clear that *all* speculation of *any* kind on My Nature is unwelcome, from both an epistemological and a Personal view. In particular, if ever again I read Professor Einstein's remarks to the effect that, whilst I am Cunning, I am not Malevolent, the extent of My lack of malevolence will be sorely tested. It is not that I disapprove of Professor Einstein's flattering description, it is that even My Patience, Infinite though It is known to be, is beginning to crack at the many, many repetitions I see of that comment. One other observation I hope never to be quoted again, at least for a long time, is Professor Haldane's, the one about the cosmos being not only stranger than one imagines but . . . you know how it goes. Whilst apposite, I feel it needs a rest.

But I digress (which is one of the qualities One finds in Omnipotence).

A periodical ostensibly concerned with matters of scientific interest would do well to think carefully before allowing speculations of a totally metaphysical nature space in which to appear. I certainly would hope that I am as disposed to be as Broad-minded as the next Entity and that no-one would accuse Me of desiring to fetter a free press, particularly in this country at this time, but I commend the matter to your most earnest consideration, if you get My drift.

I trust I shall not have to bring this to your attention on a third occasion.

God

(As revealed to Ralph Estling)

The Old Parsonage,  
Dowlish Wake,  
Ilminster,  
Somerset TA19 0NY, UK

■ It is to be hoped that putative correspondents will take the hint. Editor, *Nature*.

## Intuitive science

SIR—I have been reading *The Value of Science* by Henri Poincaré, who believed that intuition and logic make science advance. Intuitive scientists work in figures and imagination; in mathematics, they are its geometers. Logic scientists go from the particular to the general; they are the analysts in mathematics.

As a biochemist who agrees with Poincaré, I ask: what is the contribution of logicians and analysts to the advance of biochemistry? From the biochemical journals, it seems that only logicians exist. All hypotheses, even if they are subsequently shown to be wrong, are based on experi-

mental results presented in a formal and statistically rigorous way.

I believe that intuitive biochemists exist, but that their contributions have to be communicated in the form required by the journals, in analytic form. Hypotheses that are intuitive in nature are not considered suitable for biochemical journals.

Yet Poincaré points out that intuitive work is even more important than analytical work for the advance of science. In biochemistry, it sometimes seems that analytical work is performed without any precise objective. The thousands of articles in the journals contribute very little to the advance of knowledge. That is why two urgent needs in biochemistry are (1) the study of all the available information in order to advance intuitive hypotheses and (2) the recognition and promotion of intuitive biochemists through publication of that work. To these ends, journals should change their policies without lowering their quality.

Poincaré said it clearly: "Pure logic does not lead to anything but tautologies; it creates nothing new".

RAFAEL FRANCO

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## History lesson

SIR—You remark in your unsigned Commentary article marking the fiftieth anniversary of the discovery of uranium fission<sup>1</sup> that to suppose that "some history is so painful that nothing can be learned from it . . . is a disservice to the intellect" bears on three items in the previous issue of your journal<sup>2-4</sup> dealing with quark matter and strange matter. In the News and Views piece on the subject<sup>2</sup>, we find the statement that the growth phase of quark nuggets caused by the addition of low-energy neutrons "releases of the order of 20 megaelectron volts of energy yield per captured neutron . . . nearly 10 times the yield per neutron in a conventional fission reactor".

Let us hope that the human intellect has matured enough in the intervening 50 years to learn from past experience; and that if the proposal of Shaw *et al.* for quark matter engineering turns out to be successful, you will be able to write a different kind of Commentary for *Nature* in 2039.

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Italy

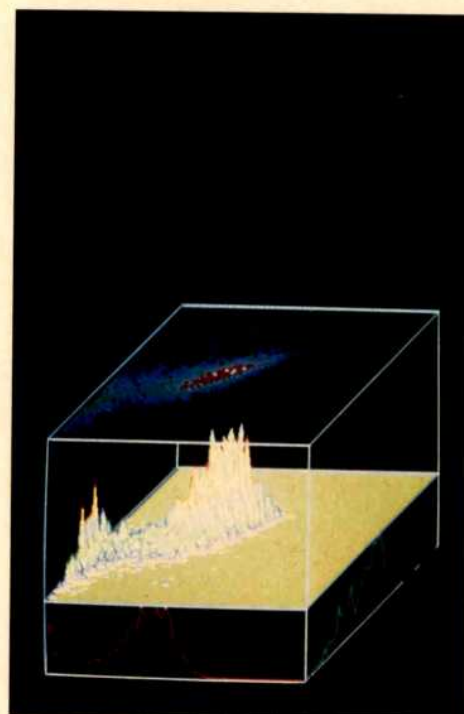
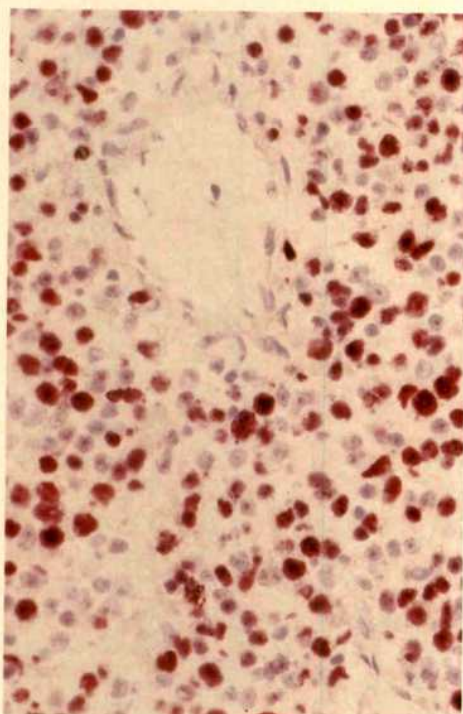
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### Mouse Anti-Human Ki-67 Antigen DAKO-Ki-67 (DAKO-PC)

**Left:**

Immunoblastic lymphoma of B cell type labelled with DAKO-Ki-67, Code No. M 722 by the APAAP procedure. The majority of the tumour cells show a positive reaction of their nuclei.



**Right:**

Con-A-stimulated lymphocytes labelled with FITC-Conjugated DAKO-Ki-67, Code No. F 788, and RPE-Conjugated DAKO-CD2, Code No. R 807 in Flow Cytometry.

Y-axis: Number of cells.

X-axis: DAKO-Ki-67/FITC.

Z-axis: DAKO-CD2/RPE.

- Reacts with a human nuclear antigen present only in proliferating cells
- Reacts with cells in late G<sub>1</sub>, S, M and G<sub>2</sub> phases, but not with cells in G<sub>0</sub> phase
- Provides a reliable means of evaluating the growth fraction of human neoplasms
- Enables a reliable identification of proliferating cells in cell suspensions by two-colour immunofluorescent staining
- Available as supernatant for immunoenzymatic staining techniques and for indirect immunofluorescence techniques, DAKO-Ki-67, Code No. M 722
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# Hot-footed towards cold fusion

The only published account so far of thermonuclear fusion in an electrochemical cell raises, as its authors say, "more questions than it provides answers".

THERMONUCLEAR fusion, which allows stars to shine, must also occur in more humdrum circumstances. Put two deuterons together in a deuterium molecule, for example, and there is a small chance that they will fuse together spontaneously, either to produce a tritium nucleus and a proton or a helium-3 nucleus and a neutron. Perhaps luckily, the chance is astronomically small, maybe  $10^{-70}$  per molecule per second. At that rate, there would be roughly one fusion event an hour in a quantity of molecular deuterium roughly as massive as the Galaxy.

The reason why muons have been advocated as catalysts of fusion is that their mass (some 200 times greater than that of an electron) makes for a smaller  $D_2^-$  ion, thereby increasing the wave function of one deuteron at the position of the other and so increasing the still-random chance of fusion.

The great excitement in the past three weeks about the prospect that thermonuclear fusion has been accomplished electrochemically is an extension of this principle. The article by M. Fleischmann and S. Pons which appeared last week (*J. Electroanal. Chem.* **261**, 301; 1989) begins from the view that conditions favourable for fusion may be created electrochemically by exploiting the familiar affinity of palladium for hydrogen (in all its isotopic forms).

As described, the experiments seem straightforward. Use a platinum anode, a palladium cathode and an electrolyte which is a heavy-water solution of deuterated lithium hydroxide. To prevent the deuterons bubbling away as deuterium, arrange that there is a substantial negative over-voltage on the cathode. The effect is that deuterons accumulate in the palladium lattice, and will continue to do so until their conversion into molecules resumes.

That point, Fleischman and Pons argue, is determined by the chemical potential of the deuterons in the palladium lattice, which is itself determined by the negative over-potential on the cathode. They use the term "galvanostatic compression" to describe this process of forcing deuterons into the palladium.

Three kinds of measurements are described, one of which is simple calorimetry. The question is whether the heat produced in such a cell is greater than expected, which is most simply that calculated

by multiplying together the current and the voltage.

The article describes measurements with cells containing electrodes of different shapes and sizes. For three-rod electrodes of different diameters, the specific rate of excess heat production is reported to have increased with increasing current density and with the thickness of the rod (4 mm at its maximum), both of which argue for a phenomenon liberating excess heat in which the bulk of the palladium is involved. There is also an account of how, in one experiment, the cathode (a 1 cm cube of palladium) vaporized.

True fusion should of course be recognizable in other ways, by the detection of its by-product particles for example. The authors report measurements of  $\gamma$ -rays presumed to have occurred by the reaction of neutrons from fusion reactions with protons in the water-bath surrounding the electrolytic cell and also the detection of neutrons (at roughly three times the intensity of the cosmic-ray background) while one experiment was running. They have also looked for (and claim to have found) tritium in the residual electrolyte in a cell.

So does this not add up to a proof of fusion? From the details published so far, no-one can say. The nuclear evidences offered are all close to the edge of what is measurable. The measurement of tritium production, for example, looks convincing, but there are pitfalls. Any quantity of deuterium-rich water invariably contains the heavier isotope in some quantity. One is looking for a small increase in tritium content, rather than its mere presence where there was none before. Similarly, there are other sources of neutrons and gamma rays besides fusion reactions (notably cosmic rays and natural radioactivity), and these must be convincingly subtracted.

Even taking the  $\gamma$ -ray and neutron production at face value, far too few of either are recorded to explain the claimed heat generation by known fusion reactions. In one of the experiments described, the rate of production of excess heat suggests that there should be between  $10^{11}$  and  $10^{14}$  fusion events a second, but the nuclear physics data suggest that known fusion reactions (leading either to tritium or  $^3\text{He}$ ) account for only about  $10^4$  of these.

This is the most serious impediment to

belief. The heat production in these electrolytic cells is more than a million times greater than nuclear by-products would suggest it can be. This leads Fleischmann and Pons to say that "the bulk of the energy release is due to a hitherto unknown nuclear process or processes". What, one wonders, can that process be? Is it likely to have escaped the attention of nuclear physicists in the past half century?

Sceptics, in the circumstances, will be quick to ask whether the necessary subtle subtractions from the gross energy output have been accurately made — the electrical energy put in, the heat that would have been produced in a normal electrolytic cell (with ordinary hydrogen instead of deuterium) and so on. Because Fleischmann and Pons spend days, perhaps weeks, loading their electrodes with deuterium, there is plenty of energy stored up in their system before any return is obtained. Even the much-described "explosion" of one of their electrodes may be explicable by chemistry.

By now, the tiny band of *Nature* referees which has scrutinized the two articles submitted for publication has been joined by many other people. Further information about the issues which have given them difficulty will be given in a further issue of *Nature*. Numerous attempts to replicate the measurements have produced only one positive claim (see page 529). One common complaint has been that the data provided are insufficient to allow faithful reproduction of the measurements reported. The Fleischman and Pons article as now published is a starting-point for other experiments, but says less than the average chemist needs to know.

A more perplexing circumstance is that the authors are reported to have said that some cells work and others do not, although that may not be surprising when so little is known of the system as a whole. The idea that cells produce excess heat only after they have been running for long periods makes sense if a palladium lattice must be charged with deuterons before fusion occurs at anything like a decent rate, but one is left wondering whether the amount of charging needed can be predicted in advance for a given cell geometry and voltage, as one would expect if such a straightforward explanation were the only one. What seems plain is that arguments like these will continue for a long time. □



# Where is the Great Attractor?

N. Kaiser

SIGNIFICANT deviations from pure Hubble expansion of the Universe have been revealed by studies of the motion of elliptical galaxies. These have been attributed to the gravitational influence of the 'Great Attractor' — an extended mass distribution whose centre lies at a distance of about  $40 \text{ Mpc } h^{-1}$  (the nearest galaxy, for comparison, is about  $\frac{1}{2} \text{ Mpc } h^{-1}$  away). The precise scale of this mass excess is the subject of intense debate, with some studies preferring a somewhat smaller distance. Two groups, however, Scaramella *et al.* on page 562 of this issue<sup>1</sup> and Lahav *et al.*<sup>2</sup>, have found a strong concentration of rich clusters of galaxies in the direction of the Great Attractor, but at the much greater distance of about  $140 \text{ Mpc } h^{-1}$ . Although the gravitational pull of these objects themselves is probably too small to account for the motions, they may be indicative of an extension of the 'supergalaxy' to a much larger scale than previously thought, with important consequences for theories of structure formation. Alternatively, the alignment may just be a chance coincidence.

The existence of a Great Attractor was initially inferred from studies of the motion of elliptical galaxies<sup>3</sup>. These revealed that galaxies in the Hydra Centaurus region, which lie near the apex of the motion of the Virgo supercluster, and so had been suspected as the cause of this motion, were themselves moving in the same direction. This could be explained by the existence of a still more distant mass concentration, dubbed the Great Attractor. Spiral galaxies also show a significant motion in this region of space<sup>4</sup>: the line-of-sight motions for a compilation of spirals and ellipticals (D. Burstein, preprint) are shown in the figure.

Much effort has been devoted to discovering the Great Attractor directly as an excess in the space density of galaxies. One approach is to use redshift surveys to map out the three-dimensional distribution of galaxies and thus infer the acceleration acting on the Local Group of galaxies. These include an IRAS (Infrared Astronomical Satellite) redshift survey<sup>5,6</sup> and Dressler's survey<sup>7</sup> which concentrates on the Great Attractor region. An

alternative is to use the much larger catalogues which have apparent luminosities but no redshift information — the idea here is that as both light and gravity obey inverse-square laws, the dipole moment of the extragalactic light should be proportional to our acceleration and thus to our motion, provided that the surveys extend far enough to encompass the source of our acceleration.

These studies seem to confirm the

attraction of matter.

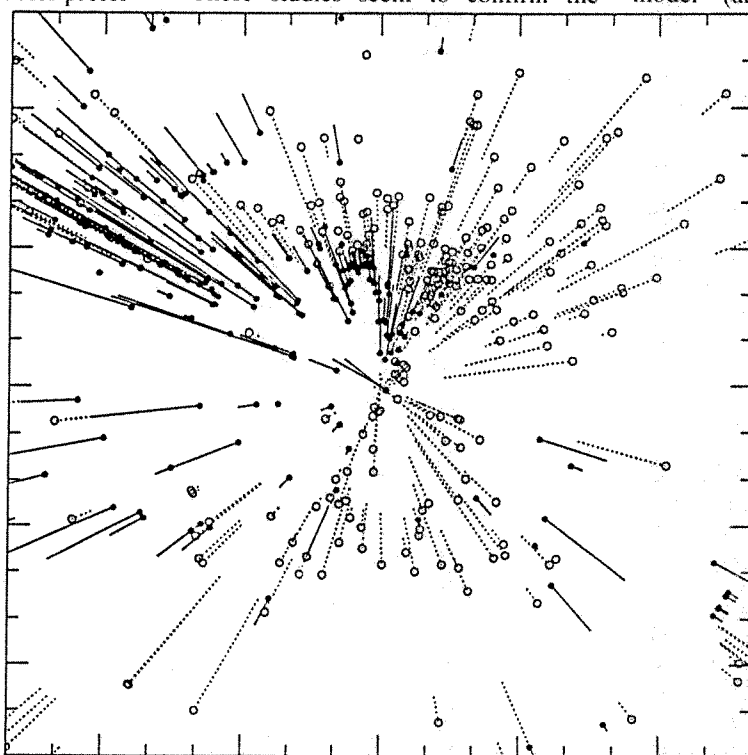
The theoretical implications of all this are less clear. A leading problem in cosmology is how the physics of the early Universe influenced the structure observed now (see also page 541). In particular, it is thought that fluctuations in the density of (perhaps unobservable) matter could have initiated the formation of galaxies and be responsible for the observed non-uniform distribution of the galaxies.

It seems, on the one hand, that the scale and amplitude of the mass profile implied by the Great Attractor are uncomfortable for the most popular cold-dark-matter model<sup>8,9</sup> (and this has stimulated interest

in alternative theories with extra large-scale power), but this is based on a rather literal interpretation of the model. The problem is that the Great Attractor model, which accounts quite well for the data, also makes predictions about the velocity field in regions where there is little or no data — on the far side of the Great Attractor for instance — so it is difficult to interpret this apparent discrepancy. On the other hand, the amplitude of the extragalactic light dipole moment agrees well with the predictions of cold dark matter.

A more recent development has been the application of correlation analysis<sup>10,11</sup>. The authors have tried to provide a measure of the scale and degree of coherence of the motions with a method similar to that used by Peeble in a powerful analysis of galaxy cluster-

ing. The conclusions here seem at first sight to be in conflict, but this is probably a consequence of the different weighting schemes used which mean that the statistics sample the velocity field at quite different distances. The less myopic sample<sup>10</sup> gives results which are not greatly out of line with the theoretical predictions. What is apparent from all these studies is that we do not yet have a sufficiently large survey to constitute a 'fair sample'.



Positions and line-of-sight peculiar velocities of local galaxies: box, centred on our Galaxy, is of side  $80 \text{ Mpc } h^{-1}$ . Hydra Centaurus lies in the upper-left quadrant, and the Great Attractor, as seen from the trend of the motions, is beyond the left edge. (Broken vectors, motion towards us; solid, away.)

existence of a significant excess of galaxies at about the right scale and direction. It is particularly impressive that the dipole moment of the extragalactic light agrees in direction with our motion to about  $10\text{--}20^\circ$ , and although there are differences — the IRAS redshift survey suggests a somewhat smaller scale for the attractor — the general impression is of a fairly consistent picture in which we can understand at least the gross features of the velocity field as the response to the gravitational

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Although the viability of the cold-dark-matter model, in the face of the velocities shown in the figure, remains very much an open question, the structures reported by Scaramella *et al.* in this issue<sup>1</sup>, which are also apparent in X-ray cluster catalogues<sup>2</sup> threaten a much wider range of theories. The major concentration of these rich clusters is so distant that it should not have contributed significantly to the acceleration inferred from either the redshift surveys or from the extragalactic dipole moment. In theories for the formation of structure which invoke gaussian random fields for the initial fluctuations that stimulated galaxy formation, one would not expect the mass excess at such distances to be aligned with the nearer mass excess, so even in theories with excess large-scale power the alignment seen is something of an embarrassment.

#### LYMPHOCYTE DIFFERENTIATION

## Not all in a name

Stephen Shaw

THE ambition of participants in the international workshops on leukocyte differentiation antigens has become nothing less than the definition and functional characterization of the entire array of cell surface molecules on human leukocytes<sup>1,2</sup>, which by the end of this year's meeting\* stood at a total of more than 78. These molecules now provide a fertile common ground, uniting the interests of immunologists, cell biologists, biochemists and those in other disciplines.

About half of the 33 newly classified molecules were previously unknown or quite obscure. For such molecules, the standardization of nomenclature and definition of reagents provides a starting point for investigation and collaboration. For some of them, the synthesis of information that occurred at the meeting was dramatic; arguably the most exciting was for a molecule now designated CD59. Based on functional, biochemical and molecular-biological approaches using two monoclonal antibodies (V. Horesji, Czechoslovak Academy of Science, Prague; G. Hale, University of Cambridge), the phosphoinositide-linked CD59 molecule is now thought to be the human homologue of murine Ly6c, a molecule capable of transmitting a T-cell activation signal<sup>3</sup>. Less direct evidence suggests that it is also identical to the molecule H19 (N4) which participates in T-cell adhesion to erythrocytes<sup>4</sup>.

The rest of the 'new' molecules were already reasonably well defined before the meeting. For some, such as LFA-3

These observations are not the first to suggest that the local supercluster is only part of a much larger structure<sup>1</sup>, and they lend support to this view. Whether this spells the end for conventional theories remains to be seen; perhaps the alignment is an acceptable chance occurrence; the X-ray cluster analysis involves a rather small number of objects, and the reliability of the Abell catalogue for these studies has recently been called into question<sup>11</sup>. Further studies are needed to establish the real extent of the supergalaxy, but have no doubt that in the meantime theorists will rise to the challenge and produce new theories to account for this tantalizing observation. □

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achieved by the workshop enriches our understanding of molecules by fostering cross-cultural exchanges of information regarding them.

In previous workshops of this series, the emphasis was largely in characterizing molecules whose expression is restricted to specific lineages of lymphoid cells such as CD3 (T3), CD19 (B4) or CD13 (MY7). In the present workshop, the emphasis shifted to molecules expressed on multiple cell types within and outside the haematopoietic lineages — not only because there are many more such molecules to define, but also because their functional importance is beginning to be appreciated. The interplay of lineage-specific molecules with molecules of broader distribution is evident in T-cell activation. The great specificity of T cells for particular foreign antigens is conferred by the T-cell receptor, a highly variable immunoglobulin-like heterodimer which associates with many other polypeptide chains to form the CD3 complex. Crosslinking of this receptor complex initiates intracellular signals including hydrolysis of phosphoinositol, Ca<sup>2+</sup> mobilization and protein kinase C activation.

An additional invariant T-cell-specific receptor, CD2 (T11), whose physiological function remains to be determined, activates a similar cascade of signals when perturbed by particular pairs of monoclonal antibodies. CD2 or CD3 generally fails fully to activate T cells by themselves; additional signals generated by other cell-surface molecules are necessary to initiate T-cell activation or to augment it. More than 15 surface molecules have been inferred to play such a role in altering T-cell activation; although many such molecules are T-cell specific (such as CD5, CD28), many others are not (such as CD43, CD45), including four newly implicated at this workshop (see table). Systematic approaches such as that described by S. Huet (Inst. Gustave-Roussy, Villejuif) promises to bring some order out of the chaos; she finds consistent patterns of complementation between particular pairs of incomplete signals mediated by CD2, CD3, CD5, CD27 and CD28.

Does this level of complexity reflect T-cell physiology, or elaborate artefact? Although the question remains open for

(CD58), ICAM-1 (CD54), and the transferrin receptor (CD71), it was a useful but unglamorous achievement to assign a standard nomenclature and to validate reference reagents. For others, such as the neural cell adhesion molecule N-CAM, ecto 5'-nucleotidase, decay-accelerating factor (DAF) and various molecules of the integrin family, it served notice that molecules being 'discovered' in one context are well known to those in other disciplines.

First, for example, CD29, used by T-cell immunologists as a marker of T-cell differentiation, is now understood to be the  $\beta$ -chain of the VLA family of integrin adhesion molecules. Second, CD56, known to immunologists as NKH1, a marker of natural killer cells, turns out to be an isoform of N-CAM. Third, many of the molecules are now being appreciated to be enzymes, including several different classes of peptidases: CD10 (ref. 5), CD13 (T. Look, St Jude Children's Research Hospital, Memphis) and CD26 (T. Mattern, Forschungs-Institut Borstel); ecto 5'-nucleotidase CD73 (L. Thompson, Scripps Clinic and Research Foundation, La Jolla); and the complement regulatory DAF protein (CD55). Thus, the unification

Molecules not restricted to T cells which are newly reported to initiate or augment T-cell activation.

CD	Other names	Reporting laboratories
CD23	B6	L. Goff, ICRF Tumour Immunology Group, London
CD54	ICAM-1	C. Cerdan, Unite 119 INSERM, Marseille
CD44	Hermes, Pgp-1, ECMRIII	S. Denning, Duke Univ., Chapel Hill S. Huet, Inst. Gustave-Roussy, Villejuif Y. Shimizu, NIH, Bethesda
CD73	ecto 5'-nucleotidase	L. Thompson, Scripps Clinic, La Jolla

\*Fourth international workshop: *Leukocyte Differentiation Antigens* Vienna, 21–25 February 1989. Organization of the next workshop is by R. Winchester and T. Springer. Summary table for antigen classification will appear in *Immunology Today* in August.

many of the molecules, biochemical mechanisms are being defined to account for regulatory effects of some of the best-studied molecules. CD5 mediates an increase in PIP2 precursor pool for phospholipase C (ref. 6), and CD28 stabilizes specific mRNA transcripts<sup>7</sup>. The 'non-linear' CD45 molecule, whose isoforms are proving to be extraordinary markers of lymphoid differentiation<sup>8</sup>, has recently been demonstrated to have an intracytoplasmic region which is a tyrosine phosphatase<sup>9</sup>. B. Schraven (Deutsches Krebsforschungszentrum, Heidelberg) reported that CD45 monoclonal antibody augments T-cell activation principally when activation occurs via the CD2 molecule, and that CD45 is physically associated with CD2, based on crosslinking studies. This finding, if confirmed, lends credibility to the model proposed by Ledbetter *et al.*<sup>10</sup> that CD45 regulates activation by association with and potentially by dephosphorylation of other receptor molecules. Together with the findings that CD4 and CD8 associate with p56 *lck* tyrosine kinase<sup>11,12</sup> (C.E. Rudd, Sidney Farber

Cancer Institute, Boston), there is potential for the complex regulation of T-cell activation by tyrosine phosphorylation and dephosphorylation. □

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## SEMICONDUCTOR CLUSTERS

# Getting smaller by design

Michael Grätzel

SUBMICROSCOPIC clusters of semiconductor material are remarkable for the unusual properties they acquire through the quantum effects of their small scale. Two groups now report unusual synthetic routes by which cadmium sulphide clusters of specific sizes can be grown. Herron *et al.*<sup>1</sup> use zeolites, porous crystalline structures, as hosts to networks of CdS clusters which they term superclusters and which have additional quantum properties. And on page 596 of this issue<sup>2</sup>, Dameron *et al.* describe yeast cells that synthesize CdS crystallites as a means to

prevent cadmium poisoning in cadmium-bearing solutions.

Colloidal semiconductor particles 1-100 nm across are a new class of materials whose properties are intermediate between those of isolated molecules and solids. Apart from their potential application in the area of photocatalysis and solar-energy conversion<sup>3</sup>, they offer a convenient vehicle for studying the transition from the molecular to the bulk state. For example, several recent studies on semiconductor colloids have shown that their optical properties depend on their size —

the so-called quantum-size effect — and are significantly different from those of the macroscopic crystals.

Size quantization effects have been widely studied in the past for metal particles. In bulk metals, considered as the limit of an infinitely large system, there is a continuous spectrum of electronic states. In contrast, in very small particles, the energy levels are quantized, the spacing between adjacent states being of the order of  $E_F/N$ , where  $E_F$  is the Fermi energy — the characteristic energy of the most significant electrons in the system — and  $N$  is the number of atoms or

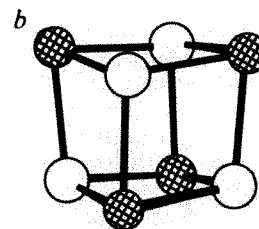
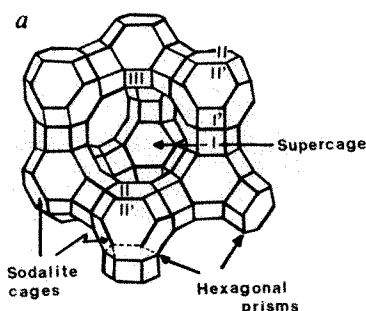


FIG. 2 a, Structure of zeolite Y and X, used in the study of Herron *et al.* b, Structure of the tetramer CdS(O) clusters located<sup>1</sup> within the zeolite/sodalite unit; (solid circles Cd, open circles S(O)).

molecules in one particle. Because  $E_F$  has a value of a few electron volts, the step size in a particle containing, say, a thousand atoms, is several millielectron volts. This quantization of states produces several anomalies in the properties of the particles, including their optical absorption, as was recognized 50 years ago.

For semiconductors, much of the earlier effort has concentrated on two-dimensional structures, such as quantum wells and superlattices. In thin films of semiconductors, a size quantization perpendicular to the film occurs. Three-dimensional systems displaying confinement effects, sometimes referred to as quantum dots, are the focus of more recent interest. Quantum-size effects in such semiconductor clusters were first observed by Berry in studies of silver halide crystals.

As the particle size is decreased, the band-edge absorption feature in the material's spectrum, corresponding to the minimum excitation, moves to shorter wavelengths (higher energies), a blueshift which is attributed to the local confinement of the electron-hole pairs (Fig. 1). This produces discrete electronic states in the valence and conduction band of the colloidal semiconductor and increases its effective band gap ( $E_g$ ). The blueshift in the absorption threshold is a consequence of the increased gap between the highest-occupied and lowest-unoccupied states. At the same time the oscillator strength of the optical transition is reduced and discrete absorption bands can appear in the spectrum. To a first approximation, the energy of the quantized levels is inversely proportional to the square of the particle diameter, because of the quantum effect

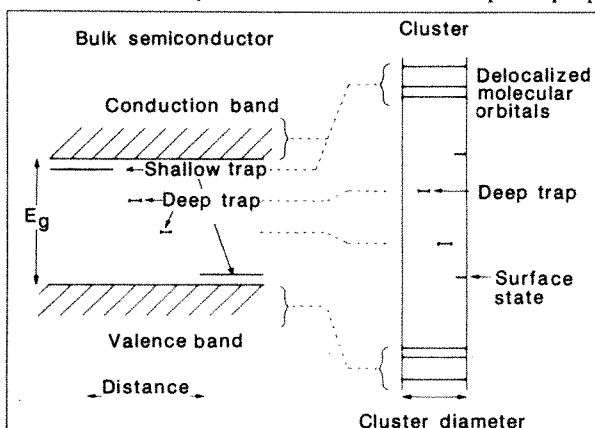


FIG. 1 Spatial electronic-state correlation diagram for bulk semiconductors and clusters. The conduction and valence bands (continuum states) of the bulk system become discrete levels because of the quantum-size effect in the cluster. Shallow and deep traps are associated with defects (impurities and lattice defects) in the crystal.



of confinement, with a correction to allow for the Coulomb interaction<sup>5</sup> between the electrons and holes. The luminescence of colloidal semiconductors is also strongly affected by particle size<sup>6</sup>. Typically, a broad emission is observed which blue-shifts with decreasing radius of the aggregate.

The synthesis of colloidal semiconductors is often carried out in solution by chemical precipitation. Because larger aggregates are thermodynamically more stable than smaller ones, it is necessary to arrest the growth process at a stage where the particles are still in the quantum-size domain. This is usually done by adding a protective agent, such as hexameta-phosphate, to the solution. However, this method gives a rather broad distribution of particle size, and tedious separation steps are required in order to obtain clusters with fairly uniform morphology.

Herron *et al.*<sup>1</sup> use an ion exchange procedure to incorporate Cd<sup>2+</sup> into the zeolite subsequently exposed to hydrogen sulphide gas to form the CdS microcrystals. An important finding from their detailed X-ray analysis concerns the location of the CdS clusters. At low concentrations, the Cd<sup>2+</sup> ions are preferentially adsorbed at the I' site of the zeolite Y (Fig. 2a), so that the clusters are located in the smaller sodalite cages and not, as one might expect, the supercages. The overall structure of the sodalite-cage-entrapped species is a distorted cube containing the tetramer (CdS<sub>4</sub>O)<sub>4</sub> (Fig. 2b). The unusual stability of these extremely small clusters is due to the coordination of the Cd<sup>2+</sup> ions with oxygen of the zeolite framework. These isolated CdS clusters are distinguished by their unique optical properties which deviate greatly from those of bulk CdS. Thus, the absorption threshold in their spectrum is around 350 nm, compared to 540 nm for bulk CdS. The absence of any luminescence emission, even at temperatures down to 4 K, is also conspicuous as large CdS particles show intense emission under similar conditions.

These optical properties undergo drastic changes at higher CdS loadings for which adjacent sodalite cages become occupied by the clusters. The absorption threshold shifts to 420 nm and an exciton-like feature (bound electron-hole 'atom') evolves at 350 nm. Strong emission appears owing to charge-carrier recombination on vacancies and lattice imperfections. These phenomena are ascribed to the formation of CdS 'superclusters' by a percolative process involving aggregation of the tetramers through the double six-rings linking the sodalite moieties. The novel feature of the superclusters is that every CdS molecule in the aggregate is on the surface and that strong coupling with the host lattice occurs through the interaction between Cd and the framework oxygen. This explains the unique optical

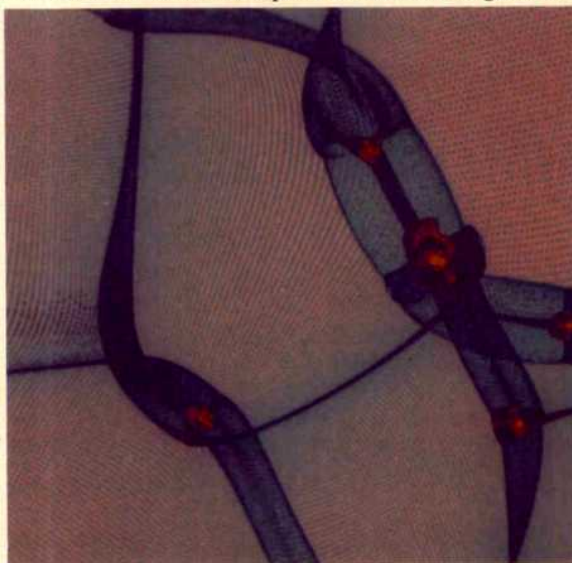
## Knotty problems in cosmology

NUMERICAL studies of the gravitational interaction of collections of point masses have been an important tool in 'experimental' cosmology for some 20 years — such interactions underlie the structure of the Universe at all scales. But many computational difficulties remain. To simulate a respectable fraction of the Universe with a limited number of particles, each particle must represent the mass of many galaxies, but now that observational cosmologists are beginning to map complete galaxy distributions throughout large regions of the Universe, the simulators can keep up their side of the work only by following their models at the level of individual galaxies. This means using enormous numbers of particles, which demands huge allotments of computing time.

A common compromise has been to put in large particle numbers, but then to approximate the behaviour of dense regions, as they form, with some kind of fluid mechanical treatment. But S. F. Shandarin (Institute of Physical Problems, Moscow) and A. Melott, working together at the University of Kansas, have returned to the simple but accurate device of calculating individual trajectories for each of 512 × 512 particles on a two-dimensional grid of comparable spatial resolution. Their technique demands too much computing power to achieve useful results in three dimensions, but reveals details which, were fewer particles used, would be invisible.

The scale of the simulations is arbitrary,

but features apparent in the figure resemble structures observed at the scale of superclusters of galaxies. The figure, a detail from one simulation, is colour coded (from red to violet) for the logarithm of the density. The darker regions could correspond to filaments of galaxies



and the intervening patches, 'voids'.

The large structures generally agree with those predicted in catastrophe theory and Zel'dovich's 'pancake' model, but the small-scale structure (such as the voids inside the filaments) is new. Of immediate interest is the suggestion that the small and large structures can be aligned over widely differing length scales. No detailed survey has yet found a scale beyond which the distribution settles to some uniform average. On all dimensions, something seems to be going on, and direct simulations such as these are needed to investigate the controlling influences. David Lindley

properties of these composites. It is noteworthy that CdS clusters in zeolite are distinguished from conventional colloids also by their selective intervention in photocatalytic processes<sup>7</sup>.

Dameron *et al.* describe in this issue<sup>2</sup> the biosynthesis of quantum-sized CdS crystallites in the yeast cells *Candida glabrata* and *Schizosaccharomyces pombe*. When exposed to cadmium ions, these cells respond with the synthesis of  $\gamma$ -glutamyl peptides, ( $\gamma$ -Glu-Cys)<sub>n</sub>-Gly and an enhancement of sulphide production. As a result, small crystallites of CdS are produced inside the cells. Surprisingly, the colloids crystallize in the rock-salt structure instead of the hexagonal configuration which is the thermodynamically stable form of CdS. The role of the peptide is that of a protective agent: the cysteinyl thiolates in the peptides are surface ligands to the core of around 85 CdS units. In this way, the organism controls particle

nucleation and growth yielding uniformly sized CdS particles of approximately 20 Å diameter. Not unexpectedly, the optical absorption of these aggregates reveals pronounced quantum-size effects as in the case of the zeolite contained CdS clusters. The findings of Dameron *et al.* provide a first example for the biosynthesis of quantum-sized semiconductor crystallites constituting a unique metabolic route for the detoxification of Cd<sup>2+</sup>-infected living cells. □

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# Pathogenesis by antisense

Bob Symons

THE first determination of a nucleotide sequence of a 7S RNA species in plants is reported in a recent issue of the *EMBO Journal* by Heinz Sanger and colleagues<sup>1</sup>. This 7S RNA, isolated from tomato leaf tissue, is very similar to the known sequence of 7SL RNA from mammals, which prompts Sanger and colleagues to suggest that the two RNAs, both 299 nucleotides long, have similar functions.

Mammalian 7SL RNA, in combination with six proteins, forms the signal-recognition particle (SRP) responsible for protein translocation after synthesis<sup>2</sup>. In their new work, Haas, Sanger and colleagues produce a computer-generated model of the secondary structure of the tomato 7S RNA and report that its two-dimensional structure is also very similar to that of the mammalian species.

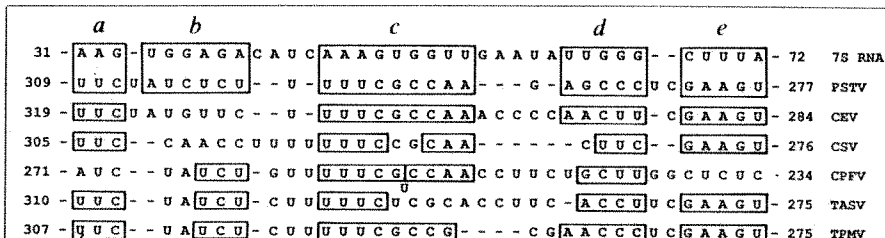
The more intriguing and unexpected aspect of the sequence and structural analysis of the tomato 7S RNA is the suggestion<sup>1</sup> that the pathogenic effects which viroids can cause in plants on infection are a consequence of the formation of a complementary hybrid between the viroid and 7S RNA. Viroids are the smallest infectious agents known<sup>3</sup>, consisting of circular, single-stranded RNA molecules which vary in length from 246 to 375 nucleotides. About 15 have been described so far, and most are pathogens that significantly affect agriculture. Many have a wide host range, infecting higher plant species, whereas others are restricted to a single family. Symptoms of infection vary

widely but the most common are dwarfing of the whole plant, curling downwards of the leaves (epinasty), and the appearance of yellow blotches on the leaves in certain hosts.

There is hardly any information as to how these intriguing agents exert their effects. One important aspect is the comparatively long time it takes for viroids to

plementary sequences in the tomato 7S RNA (see figure). The pathogenic effects of PSTV could be mediated by the formation of a 7S RNA–PSTV hybrid *in vivo* that would interfere with the normal role of 7S RNA in protein translocation across membranes. As the authors point out, viroid infection can lead to abnormal formation of cellular membranes.

Such hybrid formation would presumably need to occur in the nucleus during RNA synthesis. Although 7S RNA synthesis in tomato is likely to involve DNA-dependent RNA polymerase III, by



Possible base pairing between contiguous regions of tomato 7S RNA and of PSTV as predicted by Haas *et al.*<sup>1</sup>. Included also are possible base-paired regions in five other viroids; sequences are from refs 8, 12. Regions of three or more possible base pairs, which could theoretically form stable hybrids, are labelled A to E. There is reasonable conservation of the potential to form 7S RNA–viroid hybrids for the five boxed regions with significant variation in boxes B and C. Residue numbers given for the 5' and 3'-terminal nucleotides. G–C base pair taken as equivalent to a G–U base pair. Horizontal bars, spacings to allow maximum base pairing. CEV, citrus exocortis viroid; CSV, chrysanthemum stunt viroid; CPFV, cucumber pale fruit viroid; TASV, tomato apical stunt viroid; TPMV, tomato plant macho viroid.

produce phenotypic effects after infection, from about 2 weeks to more than 2 years. By contrast, plant viruses usually induce obvious symptoms in a few days. One of the most dramatic and damaging viroids is also the smallest: coconut cadang cadang viroid is only 246 nucleotides long, and yet it can kill coconut palms 10 to 20 metres high<sup>4</sup>. Expression of symptoms requires 2 years or more after infection, and is followed by certain death of palm trees within 5 years. This viroid is found only in the Philippines, where it destroys about 300,000 palms per year<sup>4</sup>.

Because there is no evidence that viroids can encode proteins, expression of symptoms must occur via the specific interaction of the sequence and/or tertiary structure of the viroid with one or more host components. It has been suggested by Solymosy and his colleagues<sup>5</sup> that viroids exert their pathogenic effects by interfering with the normal processing of pre-ribosomal RNA in plant nucleoli. However, the new model of Haas *et al.*<sup>1</sup> that pathogenicity results in hybrid formation between plant 7S RNA and the viroid, offers opportunities for more specific experimental testing.

In their comparative analysis, Haas *et al.* use the sequence of potato spindle tuber viroid (PSTV), which contains 359 nucleotides, as this viroid multiplies readily in tomato plants and produces characteristic symptoms of dwarfing and epinasty. They find five contiguous regions in this viroid which could theoretically form stable hybrids with com-

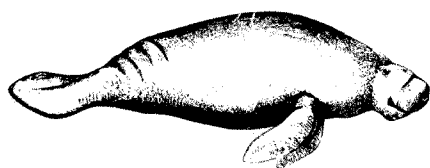
analogy with its mammalian counterpart, the RNA polymerase(s) responsible for PSTV synthesis have yet to be characterized. This viroid is known to accumulate in the nucleoli of tomato cells<sup>6</sup>, but its site of synthesis is unknown.

Direct support for the proposed model would be provided by the isolation from PSTV-infected tomato plants of stable 7S RNA–PSTV hybrids and the characterization of the base-paired regions. This would be very difficult to achieve experimentally, but supporting indirect evidence can be obtained in other ways. Haas *et al.* find that similar hybrid complexes can be formed between the tomato 7S RNA and four other viroids which infect tomato plants<sup>7</sup>: citrus exocortis viroid; chrysanthemum stunt viroid; tomato apical stunt viroid; and tomato plant macho viroid. The extra comparative data for these four viroids as well as for cucumber pale fruit viroid, which also infects tomato and produces symptoms<sup>8</sup>, are shown in the figure.

The formation and stability of 7S–viroid hybrids can readily be tested *in vitro* by hybridization of RNA transcripts, prepared from complementary DNA clones encoding tomato 7S RNA, with purified viroids. The melting profiles of such hybrids and the determination of the exact regions of hybrid formation in each of the RNA species would provide information on the ability of such hybrids to form. There may be surprises, as two-dimensional modelling cannot take into account the stability provided to an RNA–RNA

## 100 years ago

### THE MANATEE



THE Zoological Society have added to their living collection in the Regent's Park a young specimen of the Manatee (*Manatus americanus*), which those who wish to have an opportunity of inspecting an extremely curious form of Mammalian life should take an early opportunity of visiting. The Manatees belong to the order *Sirenia*, and are sometimes called "herbivorous Cetaceans," although it is very doubtful whether they have any near relationship to the true Whales or order Cetacea. These creatures were abundant in former geological epochs, but since the extermination of the *Rhytina*, or Steller's Sea-cow, at the latter part of the last century, have only two representatives still living, viz. the Manatee of America and Africa, and the Dugong of the Indian Ocean.

From *Nature* 39, 585; 18 April 1889.

hybrid by tertiary interactions. A good example of this is the very high stability of an *in vitro* hybrid formed between the 335-nucleotide linear satellite RNA of cucumber mosaic virus and part of the coat-protein gene encoding the viral RNA. Evidence indicated that the hybrid was formed by interaction between two short, separate blocks of nucleotides in the satellite RNA with two adjacent blocks in the coat-protein gene to produce a very stable pseudo-knot structure<sup>9</sup>.

Another indirect approach is to determine the sequence of 7S RNAs in other plant species where PSTV replicates and produces symptoms, and to check for the conservation of the blocks of nucleotides involved in the putative 7S RNA-PSTV hybrids. The best place to start would be with 7S RNA from two well-separated plant families, for example, another species in the family Solanaceae other than tomato, and two species from

the family Compositae which are often used for viroid replication — chrysanthemum sp. and *Gynura aurantiaca*. This approach could obviously be extended to the use of other viroids, with further fine tuning being applied through the use of different strains of the same viroid, such as PSTV or citrus exocortis viroid, where minor changes in sequence between strains can have a marked effect on the severity of symptoms in infected plants<sup>10,11</sup>.

Overall, the results of such various experiments could provide support for the model of 7S RNA-viroid interaction as the primary molecular event leading to the phenotypic disease symptoms, or require its significant modification. Whatever the outcome, the new data<sup>1</sup> make an important contribution to the unravelling of how small viroids exert their often dramatic effects. □

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## GEOCHRONOLOGY

# ESR dating for the early Earth

Rainer Grun

PARAMAGNETIC centres in crystals, revealed by electron spin resonance, are used by earth scientists and archaeologists for dating samples, but because of their short mean life, the technique is restricted to the Quaternary era — the past 1.6 million years, a mere flash in geological history. Odom and Rink<sup>1</sup> now show that two such defects in quartz can last for at least 1.5 thousand million years. Quartz being the most abundant mineral in the Earth's crust, this study lays the foundation for an exciting new geochronometer that can be used to date numerous types of geological features over nearly the whole history of the Earth.

Electron spin resonance (ESR) spectroscopy allows the detection of paramagnetic centres in crystals, many of which are created by ionizing radiation. This effect can be used in dating studies: the mineral acts as dosimeter which records all the radioactivity of the mineral itself and its environment. The number of centres of any particular paramagnetic type is proportional to the accumulated radiation dose to which the sample has been exposed since it was last 'reset'. If one can reconstruct the dose rate the sample has received, it is possible to calculate an age.

In practice the accumulated dose is determined by the additive-dose technique: aliquots of the sample are exposed to artificial  $\gamma$ -radiation so that the specific ESR signal increases. Extrapolation to zero-ESR intensity yields the accumulated dose at the intersection with the dose axis. The dose rate is deduced from the chemical analysis of radioactive elements in the sample and its environment with the influence of cosmic rays allowed for.

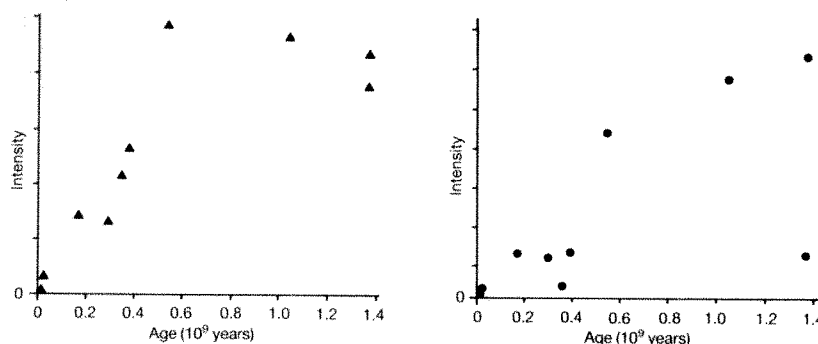
For a mineral to yield a date for an event in geological time, it must have been exposed to a process which deletes any previously stored ESR intensity. For

example, precipitation of the mineral resets the ESR clock so that speleothems, spring-deposited travertines, shells, corals or teeth can be dated. The germanium centre in quartz is bleached by sunlight, allowing the dating of dune sediments and, eventually, loess. Resetting by heating can be exploited for the dating of volcanic minerals or heated archaeological flints. And lastly, the shear stress in fault zones resets several paramagnetic centres in quartz.

As can be seen from the range of events which can be dated, ESR has a large potential for geology and archaeology. It has been applied systematically as a dating technique since 1975 when Ikeya<sup>2</sup> dated a stalactite from Akiyoshi cave in Japan. (Owing to the rapid development of the technique, the only extensive English review<sup>3</sup> is already slightly out of date and other reviews<sup>4,5</sup> have not yet been published in English.) However, when ESR is applied as a dating method as described above, the upper dating limit is set by the thermal stability of the paramagnetic centres, which persist for durations of  $10^5$ – $10^7$  years. This has restricted ESR dating mainly to the Quaternary period.

Odom and Rink now investigate two ESR centres which can be attributed to Schottky-Frenkel defects in the crystal lattice of quartz. These are created by elastic collisions of recoiling  $\alpha$ -emitting nuclei with silicon and oxygen atoms. One centre is a peroxy radical, which cannot be enhanced by artificial  $\gamma$ -irradiation, the other is the precursor to the 'E' centre, which is a  $\text{SiO}_2^+$  centre. The plot of the relative ESR intensity of these two centres versus the known age of the granites from which the quartz was separated (see figure) shows a clear relationship between these two parameters. This opens the possibility of dating quartz over nearly the whole history of the Earth.

Nevertheless, as the authors point out, several basic questions need to be thoroughly investigated. First, a precise measurement of the number of Schottky-Frenkel defects is still hard to obtain: the characteristic ESR lines are often very small and close to the background signal. Extreme spectrum accumulation or



Plot of relative ESR intensity of the peroxy-radical centre (left) and the E' centre (right) versus the known age of the quartz (after Odom & Rink<sup>1</sup>).



specifically designed microwave cavities may solve this problem. Second, the precise quantities of  $\alpha$ -particle emitting isotopes must be determined. These isotopes belong to the uranium and thorium decay chains. However, their concentration in quartz lies normally in the parts-per-billion range which is difficult to measure. The authors suggest comparing the natural ESR signal from quartz of known age with neutron-induced signals, but this approach seems unsuitable for investigation of samples of unknown age.

Third, as already pointed out by Kislyakov *et al.*<sup>6</sup>, the number of E'-centres per unit of radioactive elements may vary depending on the variety of the quartz under investigation. This dependency and also saturation effects would have to be studied. Lastly, the defects seem to be thermally stable for around  $10^9$  years. This should be confirmed, because the concentration of centres reaches a steady state in samples older than the mean life of the defects. It was previously reported<sup>7</sup> that the point defects which

lead to the E' paramagnetic centre have a mean life in the range of  $10^8$  years (at  $20^\circ\text{C}$ ).

Although the investigation of these problems is not a trivial task, it seems now very likely that in the near future, ESR dating can be extended from the Quaternary to the whole geological timescale and that ESR dating will be applied to many chronological problems which could not be solved with established dating techniques. □

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## CONSERVATION BIOLOGY

# Nine hundred kiwis and a dog

Jared M. Diamond

INTRODUCED predators are thought to be a leading cause of the extinction of island species. Yet the evidence for this is inferential in many cases, with few or no direct observations of predation. It remains baffling how victim species can disappear so quickly, and how a predator species can eradicate a victim species completely before its own numbers collapse. Thus, the recently documented collision between one dog and a population of 900 kiwis is likely to become cited as a classic case study in conservation biology (Taborsky, M. *Notornis* **35**, 197–202; 1988).

New Zealand has lost a high proportion of its native bird species in the few centuries since Europeans arrived. Introduced weasels, cats and dogs may be responsible, but firm proof is lacking even for well-known endangered species such as the kiwi, New Zealand's national bird. The three species of kiwi are all large, flightless birds confined to New Zealand and adjacent islands, but it is not known why one (the little spotted kiwi) has vanished from the New Zealand mainland.

Until recently, the 11-square-mile Waitangi State Forest supported the largest-known and counted population of brown kiwis (*Apteryx australis*). Between June and October 1987, biologists marked some individuals of this population and followed them using radio transmitters.

On 24 August 1987, a large female kiwi was found freshly dead. Over the next 6 weeks more fresh carcasses were located, usually buried under leaves and soil. Of the 23 marked birds, 13 died during this period and were discovered through their radio transmitters. Despite the very low chances of detecting a buried kiwi carcass without the help of a transmitter, 10 carcasses of unmarked individuals were



The brown kiwi is defenceless against introduced predators.

also discovered. By the end of September many fewer kiwi calls were being heard, and a dog trained to locate kiwis (but not kill them) could find none.

Tooth marks and footprints showed that all the dead kiwis had been killed by a dog, which had buried but not eaten them. All parts of Waitangi Forest that were searched yielded dead kiwis, dead possums, dog footprints, or dog faeces

containing kiwi feathers or possum remains. The footprints and faeces all appeared to be from the same dog. On 30 September, a German shepherd dog was found in the forest and shot. Thereafter no marked kiwis disappeared and no further dog signs were found.

How many kiwis did the dog kill? The 13 deaths among the 23 marked birds would mean 500 deaths in the whole population of 900, if marked and unmarked birds were at equal risk. In fact, the population may have suffered greater losses, because the proportion of marked birds killed was nearly twice as high outside than inside the main study of 0.5 square miles, where the biologists' presence may have deterred the dog. Five hundred killings in 6 weeks would mean about 12 kiwis killed per night. That kill rate may at first appear unbelievably large, but kiwis have a very strong distinctive smell, forage and call noisily, so are particularly vulnerable to predators.

The Waitangi incident illustrates many points relevant to the extermination of island species by introduced predators. Kiwis evolved in the absence of mammalian predators and are entirely defenceless against dogs. They breed so slowly that the Waitangi population will require an estimated 8–20 years to recover even if no such incident recurs. It took just one dog to halve the size of the largest-counted brown kiwi population in 6 weeks, yet the cause of that decline would have remained unknown but for the coincidence that some of the buried carcasses had been radio-tagged. Hence it becomes less surprising that New Zealand's abundant feral cats and weasels could have exterminated other native species without being identified as the culprits.

The kiwi incident also illuminates the paradox of how a predator could exterminate its prey without exterminating itself first. Like most other introduced pests, dogs are 'switching predators' for which kiwis are just one of several potential prey species: this particular dog did not even eat the kiwis that it killed.

Finally, the Waitangi incident emphasizes the importance of control of introduced pests. Responsibility for the forest was recently transferred from the New Zealand Forest Service to a timber corporation, which abandoned the previous policy of keeping the forest free of dogs. As Taborsky says, "The disappearance of kiwis from other populated parts of New Zealand during the last decades underlines the general importance of the issue to the protection and management of kiwis". □

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## NITROGEN FIXATION

# New route to a sticky subject

Sharon R. Long and David W. Ehrhardt

RECOGNITION between cells or organisms is an essential component of pathogenesis, of defence and of development itself. All three processes are involved in the nitrogen-fixing symbiosis between *Rhizobium* bacteria and host plants. Specificity is one of the most prominent features of symbiotic nodule development: particular bacteria will invade and form nodules only on some host plants and not others. Because it seemed logical that surface interactions might be involved, much attention has been given to the notion that plant proteins such as lectins might specifically bind to bacteria surface determinants<sup>1</sup>. It has been a difficult and so far elusive task to identify the bacterial surface components that might be responsible for the host-specific interaction of *Rhizobium* with plants. Now, a new study reported by Díaz *et al.* on page 579 of this issue<sup>2</sup> uses a fresh route to test specificity determinants: the genetic alteration of the composition of the root.

The authors introduce a lectin gene from pea (*Pisum sativum*) into the genome of clover (*Trifolium repens*) roots via *Agrobacterium rhizogenes* transformation. The transgenic roots still nodulate with the clover symbiont, *Rhizobium leguminosarum* biovar *trifolii*, but they display an elevated frequency of nodule formation with *R. leguminosarum* biovar *viciae*, the pea symbiont.

Host range in the *Rhizobium*-legume system exists at several levels. First, the Rhizobiaceae (*Rhizobium*, *Bradyrhizobium* and *Azorhizobium*) generally infect only plants in one plant family, the Leguminosae. Second, there are 'cross-inoculation groups' of *Rhizobium* and plants, defined by sets of bacterial strains (formerly considered species) which typically nodulate the several members of a corresponding plant set. *R.l.* biovar *viciae*, for example, nodulates peas, vetch and a few other plants, but not clover. (The study by Díaz *et al.* concerns the basis for this cross-inoculation group level of specificity.) Finally, there is a fine structure to nodulation host range, such that certain strains of a bacterial species might not nodulate all genotypes of their usual host; this behaviour is sometimes conditioned by single genes in the bacterium and the host plant.

A successful combination of host and bacteria requires compatibility at many different stages in the symbiosis, from the initial interaction to successful differentiation to nitrogen fixation<sup>3-5</sup>. The success of early *Rhizobium*-host interactions is characterized by the curly deformation of the plant's epidermal root hairs, the invasion of these root hairs by bacteria

within an infection thread (see Fig. 1) and the initiation of new cell divisions within the plant root, which eventually result in the outgrowth of a nodule. Because host specificity is often limited at these early steps, they have received most attention.

Bacteria and plants that are not in the correct cross-inoculation groups — such as *R.l. viciae* placed on clover plants — do not get as far as the infected root hair shown in Fig. 1; so there must be some barrier early in the interaction. Attachment of bacteria to the root surface might be an essential step, and it has been proposed that lectins could mediate this interaction<sup>6</sup>. Attachment, however, is hard to quantify, because the highly charged cell wall that surrounds all plant cells gives a substantial background to measurements of bacterial binding. Binding assays carried out using microscope observation have not been uniformly correlated with quantitative measurements using radioactively labelled cells<sup>1</sup>. The strength of the study by Díaz *et al.*<sup>2</sup> is that such a problematic assay is not involved; a discrete change was made in host cell determinants using the plant's own cellular machinery, and this change

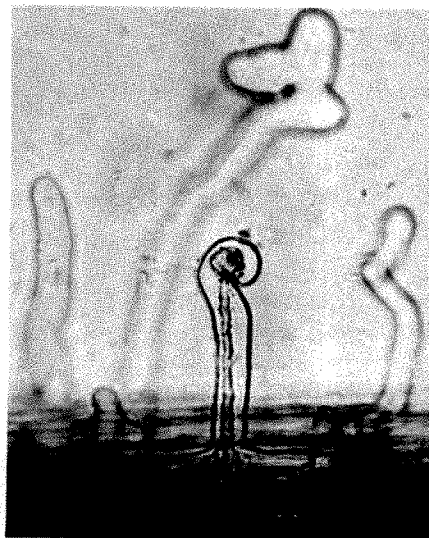


FIG. 1 Successful infection of *Rhizobium* into many legumes is characterized by formation of an infection thread in an epidermal cell. Normally, straight root hairs grow into a curled form and are invaded by *Rhizobium* of the correct cross-inoculation group. No defence reaction occurs in the root-hair cell; instead, the bacteria penetrate to the main body of the root within the actively elongating, branching infection thread whose wall is synthesized by the plant cell. The dynamic process must involve highly localized and directed bacterial functions, and a complex cell-wall growth response in the plant. Genetic studies on *Rhizobium* indicate that specificity determinants are an intrinsic part of the mechanisms directing these active plant responses.

leads to a marked alteration in nodulation phenotype. It is noteworthy, however, that although the transgenic clover roots studied here gain significant nodulation functions, the gain of function is not complete. Nodules form infrequently, and are often abnormal in morphology, suggesting that although the pea lectin represents one primary limitation or screen for symbiotic partners, there are others.

That the determination of host range involves multiple components and steps is also now indicated by studies of bacterial symbiotic genes. Numerous bacterial genes have been identified as affecting nodule formation, and these fall into several categories<sup>3-5</sup>. Some, for example *nodABC*, are highly conserved, both in DNA sequence and in function, and seem to be central to the stimulation of nodule formation on all plants. Another type, such as *nodH* in the alfalfa symbiont *R. meliloti*<sup>7</sup>, are unique to bacteria of a particular nodulation specificity. Other genes, including *nodFE*, are conserved among different *Rhizobium* strains in terms of their nucleotide sequences, but they nevertheless seem to affect host range. The *nodD* regulatory genes even seem to combine universality and host selectivity: although the ultimate regulatory effect of *nodD* — the activation of transcription of nodulation genes — is parallel in various *Rhizobium* strains, the spectrum of plant-derived inducer molecules which stimulate this effect varies for *nodD* genes derived from different *Rhizobium* species<sup>8</sup>.

The genes known to be present in *R.l. trifolii* and *R.l. viciae* are remarkably similar (see Fig. 2), yet the bacteria nodulate different hosts<sup>9</sup>. Experiments to analyse this have revealed unexpected properties of some host-range genes. *R. l. trifolii* containing a mutated *nodF* or *nodE* gene is converted to behave more like *R.l. viciae*, for example — it broadens its host range to interact with pea, and becomes less efficient on its original host, clover<sup>4</sup>. In general, individual host-specific nodulation genes seem to affect efficiency or frequency, but do not seem to be absolutely required. Thus, whereas host range itself is a strict phenotype, mutation of individual bacterial genes controlling host range gives leaky phenotypes. Such observations, together with studies in several laboratories of double mutants, have led to the widely discussed proposal that the host-range phenotype results from the additive effects of multiple mechanisms, which may exist in differing combinations among various host-microbe pairs, not in mutually exclusive sets. The picture that has emerged from genetic studies, then, is that host range is not controlled by a single locus, in which one key is matched to a lock, but is more likely to be combinatorial.

Díaz *et al.*<sup>2</sup> demonstrate that a lectin can

FIG. 2 Map of nodulation genes in two biovars of *Rhizobium leguminosarum*, *a*, by *viciae*, which infects pea, vetch, lentil and related plants; and *b*, by *trifolii*, which infects clovers. The genes have been established by transposon mutagenesis, sequencing and some protein product analysis<sup>4,5</sup>. Asterisks indicate positions in which various researchers believe further genes will soon be defined. Those genes designated 'common' are functionally interchangeable without an effect on host range; in particular, *nodABC* seem to stimulate host cell divisions and nodule formation, and are also required for root-hair deformation<sup>3</sup>. These common genes do not function well without accompanying host-specific nodulation genes, such as *nodFE*, which appear critical for proper root-hair curling and infection<sup>4,5</sup>. An example of a fine-structure host range gene is *nodX*, found in some but not all *R. l. viciae*; without *nodX*, *R. l. viciae* strains are restricted to nodulating only certain genotypes of pea<sup>5</sup>.

Host plant specific	"Common"	Host genotype specific
* NM L EF D ABCIJ X		<i>R. l. viciae</i>
* NM L* EF D ABCIJ *		<i>R. l. trifolii</i>

be important in this process. Whether changing the lectins expressed in other host plants can similarly affect infection by *Rhizobium* remains to be seen. The two bacteria which nodulate pea versus clover are more closely related than most pairs of *Rhizobium*. Those which are less closely related may have more dramatic limitations to nodulation on their heterologous hosts. It would not be surprising if different host/symbiont combinations in varying isolation evolved exclusivity and compatibility to different degrees and along slightly different avenues.

The molecular role of any receptor, of course, remains difficult to study until it is known to what it binds. This remains a sticky point in research on *Rhizobium*-legume systems. If plant lectins are receptors for bacterial carbohydrates, it may be that the relevant sugars are sweetening up a surface component not identified to date. For example, researchers working on the genes which control several types of extracellular polysaccharides in *Rhizobium* find that eliminating or altering surface components such as the acidic exopolysaccharide<sup>9</sup> or the lipopolysaccharide<sup>10</sup> prevents successful invasion, but allows formation of uninfected nodules in a correct host-specific manner. Therefore, these carbohydrates do not seem to correlate with host range, although it remains possible that there is redundant information in several surface determinants. Identification of specific bacterial molecules interacting with host components will be essential before the issue of how lectins affect the symbiosis is resolved.

The availability of transgenic roots with altered nodulation properties will allow genetically tailored microbes and plants to be paired with each other for specific mechanistic tests. It is of interest, for example, to test bacteria altered in known nodulation genes on transgenic roots with host-range changes that are complementary. Expression of lectin genes in heterologous backgrounds may help to determine exactly where the lectin is expressed and localized in transgenic plants, for example, and under what circumstances its functional properties are preserved. Previous studies on pea have shown that lectin binding of *R. l. viciae* can be assayed only under manganese limitation of the

bacteria<sup>11</sup>: do the transgenic clover/pea lectin roots bind their new *R. l. viciae* symbionts in this manner?

Finally, how are host-range functions coupled with the action of the common nodulation genes? Genes such as *nodABC* drive the plant to begin cell divisions, and are also required for root hair curling and invasion, but they do not have a productive effect on a host plant unless they are accompanied by the proper host range genes. Bacteria with normal *nodABC* genes, but lacking a host-range gene, for example, often cause the plant to exhibit incomplete or atypical root-hair deformations. If host-range genes are additive in effect, then perhaps they act by modifying a central function or functions provided by the common *nod* genes, increasing their efficiency. There is new experimental support for this model<sup>12</sup>. In addition, host-range genes might also act by directing the location and timing of the action of *Rhizobium* on its target; by providing access for a molecular signal or a cell to a site of action; by actively modifying the bacteria's immediate molecular environment within the plant cell wall or membrane; by preventing a host defensive reaction; or by some combination of these. Given that the transfer of the pea lectin can change the responsiveness of clover roots to *Rhizobium*, it may be possible to test whether the function of this lectin is simply in attachment, or whether it acts through more complicated, possibly more active, mechanisms. □

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## Long sight

LAST week Daedalus devised his 'Space Spider'. This ingenious spacecraft propels itself by melt-spinning and ejecting a fine optic fibre. Cunningly, the fibre is made of a lasing glass, excited by the sunlight of space. It amplifies light pulses as they traverse it, and forms an active optical communication link back to Earth.

Daedalus now reckons that his space-borne light guide should be a powerful research tool in its own right. Thus it could form an astronomical interferometer of unprecedented resolution. The light from a stellar source spreads out in a wide wavefront. When two separate sections of the wavefront are recombined, traditionally by means of a pair of mirrors, the resulting interference pattern can be decoded into an image of the source. It is hard to give such an interferometer a mirror separation greater than about ten metres, so it can only just resolve the diameters of the nearer, bigger stars. But an active space-borne optic fibre could recombine sections of wavefront millions of kilometres apart.

Daedalus envisages a command spacecraft with two fibres extending out from it, their far ends anchored to two observation platforms. Each platform would use a stabilized telescope to image light from the chosen astronomical object onto the end of its fibre. The light from the two telescopes would flow down the fibres and interfere at the central command craft. If the orbits of the telescopes were chosen to vary the distance and angle between them, the changing interferogram would slowly accumulate an aperture-synthesized image of the object. A source a thousand light years away could be resolved to a few kilometres!

More boldly still, Daedalus wants to use his space-borne fibre to detect gravity waves. These energetic but elusive phenomena are emitted by massive moving objects like big, short-period binaries, collapsing neutron stars, supernovae, and black holes in the act of swallowing a star. Even gravity waves from the early days of the Universe may still be echoing around. They should shake an optical interferometer as they pass through it. Terrestrial interferometers have so far failed to detect them: but Daedalus's space-borne fibre interferometer should be mightily more sensitive. Its optical length would only change in second order, but the shift of the interference pattern should still be very clear. Gravity waves would also deform the fibre slightly as they passed through it. The birefringence thus induced would be tiny indeed — but a light pulse traversing a million kilometres of such a fibre should suffer detectable changes of polarization. Much exciting astrophysical mayhem, undetectable by optical or radio emission, should thus be revealed in all its gravity.

David Jones

# Leucine zipper motif extends

SIR—Landschulz *et al.*<sup>1</sup> have recently proposed that the 'leucine zipper' is characteristic of a new category of DNA-binding proteins. In this hypothetical structure the side-chains extending from an heptadic repeat of leucine residues over a distance covering (at least) eight turns of an  $\alpha$ -helix, interdigitate with those on a similar helix of a second polypeptide, thus facilitating dimerization. The Fos and Jun transforming proteins both contain such a motif and Landschulz *et al.* speculate that it plays a role in the heterotypic complex these proteins can form<sup>1</sup>.

We report the presence of the same motif in proteins that are not DNA-binding. As shown in the figure, the fusion (F) glycoproteins of paramyxoviruses each have a leucine-zipper motif, which is in an  $\alpha$ -helical structure situated 4–11

Virus		Ref.
MV	NLGNALAKLEDAKELLESSDQILR 7	
RPV	NLWNAVTKLEKAKDLDDSSDLILR 8	
CDV	NLGNALKKLDDAKVLIDSSNQILE 9	
HPV 1	NLASATNPLQQSKIQLMKAKAIIIS 10	
HPV 3	ELNKAASDL EE SKEWIRRNQKLD 11	
Sendai	NLADATNPLQDSKAELEKARKILS 12	
Measles	ELSKVNASLQNAVYIKESNNHQLQ 13	
SV 5	NLAAVNKSLSDALQHLAQSDTYLS 14	
NDV	ELGNVNNISNALDKLEESNSKLD 15	

Comparison of leucine-zipper motif in F proteins of different paramyxoviruses. Leucine heptad repeats are marked by asterisks. Viruses: MV, measles; RPV, rinderpest; CDV, canine distemper; HPV 1, human parainfluenza 1; HPV 3, human parainfluenza 3; SV 5, simian 5; NDV, Newcastle disease.

amino acids from the transmembrane area. In some cases, isoleucine is substituted for one of the leucines, but Kouzarides and Ziff<sup>2</sup> have shown that this does not affect the interaction of Fos and Jun. Further analysis of the proposed structure reveals that at position 4 alongside the leucine, there is always a small uncharged amino acid, whereas charged amino acids are distributed in the other five positions.

Studies with influenza haemagglutinin<sup>3</sup> and the glycoprotein of vesicular stomatitis virus<sup>4</sup> have shown that after their synthesis in the endoplasmic reticulum, these glycoproteins must oligomerize in order to be transported to the cell surface via the Golgi complex. Conceivably, the same is

true for the F glycoproteins of paramyxoviruses, in which case dimer and tetramer formation may be mediated by the leucine zippers. The F glycoprotein of Sendai virus is a tetramer when isolated under non-denaturing conditions<sup>5</sup>.

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## Stereo problem

SIR—I have frequently suffered frustration because of the reversed depth and handedness of stereo-images when viewed directly from the printed page, especially when other clues such as the size of components or their shading are adjusted to give parallel depth information. Like most people, I view such images with crossed visual axes. The diagram in Vance Tucker's letter (*Nature* 337, 605; 1989) suggests a simple solution.

If one member of a stereo-pair were to be flanked on either side by identical copies of the other stereo-pair, as in Tucker's diagram, then a reader could see the correct depth and handedness by looking at, say, the left pair with uncrossed visual axes and at the right pair with crossed axes. The same procedure could also be used with projected images from slides, films or overhead projectors, although given that many individuals who view close stereo-pairs with uncrossed axes view distant pairs by the crossed method, it would probably suffice to project only the 'crossed' arrangement. Unfortunately, in my experience it is more usual for the uncrossed images to be projected or printed (for example, the stereo-pairs on pages 618 and 619 of the same issue of *Nature*), causing confusion to most direct viewers.

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## Stop taxonomists

SIR—Replying to complaints<sup>1</sup> about the number of changes in biological nomenclature, Hawksworth suggests<sup>2</sup> that "international peer reactions are the most likely prospect for restricting unwelcome changes...". Unfortunately, this restriction seems unlikely to happen if the peers are fellow taxonomists.

One difficulty stems from some of the general assumptions of taxonomists: even Hawksworth's distinguished predecessor, G.C. Ainsworth, held that more is better.

He wrote<sup>3</sup>: "the very welcome upward trend in the proportion of new combinations indicates that increasing attention is being paid to revision".

Biologists need good taxonomy, with each name unambiguous and reasonably constant. However, in their enthusiasm, taxonomists make frequent changes and revisions of these changes that cause confusion to other biologists who use the names. This is nothing new; it has been going on for years and it is time it stopped.

This conflict of interest between taxonomists, who want to change the names of organisms, and other biologists, who do not, could be resolved. Taxonomists could continue publishing their findings, as now, but international standing commissions could review these publications every few years and issue lists of official names. These names would hold until the next list was issued, so that names would be standardized over periods of several years.

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## Carbonatite origin and diversity

SIR—A recent paper<sup>1</sup> and an accompanying News and Views article<sup>2</sup> explained the origin and diversity of carbonatites by production of primary carbonatite magma in the mantle, followed by fractional crystallization. Gittins<sup>2</sup> considers the experimentally produced carbonatite melt of ref. 1 to be particularly relevant to the problems of carbonatite magma genesis because it contains the oxides required to crystallize the silicate, phosphate and oxide minerals that are commonly associated with the carbonate minerals that form the majority<sup>3</sup> of carbonatite rocks.

In contrast, Gittins<sup>2</sup> asserts that the carbonate liquids produced by immiscibility in our experimental studies<sup>4,5</sup> are too poor in non-carbonate components to be considered relevant to natural systems. Twyman and Gittins<sup>6</sup> previously postulated a mildly alkalic olivine sövite as a suitable parent magma; the proposed composition of this magma is listed in the table, together with the carbonatite melt composition of ref. 1 and immiscible silicate/carbonate liquid pairs from refs 4 and 5 and our new work (in preparation).

Contrary to Gittins' statement, some of the carbonate liquids produced experimentally in ref. 4 contained significant amounts of non-carbonate components. Although the FeO and MgO contents of these carbonate liquids are quite low, this simply reflects the fact that the starting bulk composition was very poor in these

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Composition of carbonatite melts and conjugate silicate liquids

Oxide	1	2	3	4	5	6	7	8	9	10
SiO <sub>2</sub>	2.94	6.56	4.50	1.60	6.43	9.33	44.46	32.38	42.12	32.51
TiO <sub>2</sub>	0.45	1.66			0.94	0.81	0.73		2.28	1.70
Al <sub>2</sub> O <sub>3</sub>	1.95	1.09	0.40	0.36	0.80	0.91	14.68	8.62	14.79	7.76
FeO <sub>tot</sub>	4.61	10.66	2.30		4.18	4.12	5.41		7.92	6.65
MgO	14.19	3.68	0.92		1.00	10.93	0.65		0.91	10.49
MnO		0.25			0.23				0.18	
CaO	21.29	34.50	31.59	55.46	37.16	29.19	11.13	42.92	12.77	16.14
Na <sub>2</sub> O	4.99	5.74	16.35	0.28	7.27	3.56	11.07	6.22	8.91	6.60
K <sub>2</sub> O	0.35	2.41	5.17		1.73	2.34	5.73		3.53	3.34
P <sub>2</sub> O <sub>5</sub>	0.48	5.92	1.21		0.56	2.19	0.23		0.12	1.41
CO <sub>2</sub>		28.17								
Total	51.49	100.00	62.44	57.70	60.30	63.37	94.09	90.14	93.53	86.60

- (1) Carbonatite melt produced in equilibrium with a peridotite source (column 3 of Table 1 in ref. 1). (Total includes Cr<sub>2</sub>O<sub>3</sub> = 0.22.)  
 (2) Carbonatite parent magma of Twyman and Gittins<sup>6</sup>.  
 (3) Immiscible carbonatite melt FH24 of Freestone and Hamilton<sup>4</sup>,  $P = 7.6$  kbar,  $T = 1,100$  °C.  
 (4) Immiscible carbonatite melt KH11 of Kjarsgaard and Hamilton<sup>5</sup>,  $P = 5.0$  kbar,  $T = 1,250$  °C.  
 (5) Immiscible carbonatite melt BK208 of Kjarsgaard and Hamilton (unpublished),  $P = 5.0$  kbar,  $T = 1,000$  °C.  
 (6) Immiscible carbonatite melt BK193 of Kjarsgaard and Hamilton (unpublished),  $P = 6.0$  kbar,  $T = 1,200$  °C.  
 (7) Conjugate silicate liquid to (3).  
 (8) Conjugate silicate liquid to (4).  
 (9) Conjugate silicate liquid to (5).  
 (10) Conjugate silicate liquid to (6).

components. The distribution of elements between immiscible liquids is strongly affected by pressure, temperature and the bulk composition of the system<sup>4</sup>, with compositional effects being particularly important (see Fig. 6 of ref. 4). Kjarsgaard and Hamilton<sup>5</sup> used compositions containing only five components (SiO<sub>2</sub>-Al<sub>2</sub>O<sub>3</sub>-CaO-Na<sub>2</sub>O-CO<sub>2</sub>), so the immiscible carbonate liquids produced could not contain FeO, MgO, P, O<sub>2</sub> and the like. Gittins was correct to point out that these melts were poor in Si O<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> (see column 4 of the table), but again this could be the result of pressure, temperature and composition. Our more recent (unpublished) results show that the addition of TiO<sub>2</sub>, MgO, FeO, F and P<sub>2</sub>O<sub>5</sub> ensures that these components occur in the immiscible carbonate melt, and also that the addition of these components affects the partitioning of Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub> between immiscible melts such that the carbonate liquids contain noticeably higher contents of these two elements.

The table shows that we have produced, by immiscibility, carbonate liquids whose compositions compare favourably with those considered to be "realistic carbonatite parent magmas" by Gittins (compare columns 5 and 6 with columns 1 and 2). Furthermore, the conjugate alkali silicate liquids produced in these experiments (compare columns 9 and 10) have compositions that are suitable as parent magmas for the attendant alkali silicate rocks found in carbonatite complexes.

Gittins<sup>2</sup> suggests that the next step in testing the primary-melt model of ref. 1 is to examine rare-earth element concentrations. Hamilton *et al.*<sup>7</sup> have made this test for an immiscible silicate/carbonate system, using lavas from Oldoinyo Lengai as

starting materials. Rare-earth element concentrations for the Oldoinyo Lengai phonolite and natrocarbonatite<sup>8</sup> are used in ref. 7 to calculate a set of natural rock partition coefficients, and these compare very well with those derived from the experiments at 3 kbar and 1,050 °C.

Any debate on carbonatite magma genesis should include proper consideration of the viability of the immiscibility hypothesis. This process, in conjunction with fractionation, can convincingly account for both the major- and trace-element contents of carbonatites as well as the associated alkali silicate rocks.

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GITTINS REPLIES—The proponents of liquid immiscibility as the explanation for carbonatite magmas consider the experimental demonstration that various carbonate and silicate liquids are immiscible up to at least 8 kbar pressure to be proof that the carbonate and silicate liquids separated from a common parent magma, whereas I maintain that one must demonstrate not only the existence of liquid immiscibility but also a derivative relationship. In my view this requires the formation of a single quenchable liquid from the two immiscible liquids. Such a liquid might be the hypothetical CO<sub>2</sub>-rich nephelinite (or phonolite) that is invoked by Le Bas<sup>9</sup>. If it exists in nature it should be possible to make it in the laboratory.

Until this is done we can only examine the natural rocks for evidence of a CO<sub>2</sub>-rich nephelinite that has fractionated to a stage just short of single-liquid instability,

and ascertain what sort of rock it is. We find no such rock — only simple olivine nephelinites and nephelinites.

The postulated parental magma of ref. 6 is not completely correct (for example, the P<sub>2</sub>O<sub>5</sub> content is too high) but it is a useful beginning. Some of Kjarsgaard and Hamilton's immiscible carbonate liquids are similar but they and the  $K_D$  values of the table are relevant to the problem only if carbonatite magma separates immiscibly from a silicate parent (which is not yet proved) and if the contents of rare earths and other elements such as Nb, P, Sr, Ba, F and Cl are sufficiently high in the parent silicate magma for partitioning to produce the characteristic carbonatite element concentrations. In saying that the next step in testing the primary melt model of ref. 1 would be to examine the rare-earth element concentrations, I was simply making the point in my News and Views article<sup>2</sup> that unless the liquid of ref. 1 contains the necessary major- and trace-element concentrations it is not a viable parental carbonatite magma. The fact that Hamilton *et al.* have successfully partitioned rare earths into a carbonate liquid at the expense of an immiscible silicate liquid is interesting, but irrelevant to evaluating ref. 1.

I might also point out that if the experiments in ref. 7 used only CO<sub>2</sub> as a transporting fluid they are of limited value because the mobility of CO<sub>2</sub> is restricted to a depth corresponding to 27 kbar, the pressure at which silicate carbonation reactions fix CO<sub>2</sub>. Something capable of transporting elements at greater depths is required, and our (unpublished) work suggests that fluorine plays a major part in this and in just about every other aspect of carbonate liquid systems.

Carbonate/silicate liquid immiscibility exists. But is it merely a physical property of silicate and carbonate magmas or does it control their genesis? Do these magmas separate as conjugate liquids from a single mantle-derived parent, or are they each the result of separate mantle-melting events? Is immiscibility a relatively late-stage, upper-crustal process of minor petrological significance, or the principal process by which carbonatite magma is generated?

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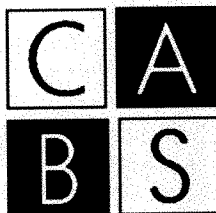
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# In pursuit of the peculiar

P.V.E. McClintock

**Methodological Aspects of the Development of Low Temperature Physics 1881–1956: Concepts out of Context(s).** By Kostas Gavroglu and Yorgos Goudaroulis. *Kluwer Academic*: 1989. Pp. 178. Dfl.125, \$67, £36.

PROGRESS in science often arises through a fruitful interplay between experiment and theory. New experimental observations — made possible, perhaps, by some advance in instrumentation — may fail to agree in all respects with theories constructed to account for related phenomena; those theories then have to be modified, leading to fresh insights and, perhaps, to new predictions which can then in turn be tested. But what happens when the discrepancy between observation and expectation is so gross that the existing theoretical framework simply cannot, by any stretch of the imagination, be modified or extended to accommodate the new information? In their book, Gavroglu and Goudaroulis attempt to answer the question through an historical study of low-temperature physics.

Both of the authors are active in theoretical atomic and elementary particle physics. Given this background, it is interesting to note that, in seeking the “most peculiar” phenomena on which to base their study — admittedly a subjective judgement — they eventually “were amazed to realize that neither the phenomena of the very small nor those of the very large could compete with the phenomena of the very cold”.

The phenomena in question are superfluidity and superconductivity, and they certainly are peculiar. Each of them involves the onset of a form of frictionless flow below a critical temperature. In the case of helium, the liquid acquires the ability to flow easily through tiny orifices of vanishingly small dimensions or, even more astonishingly, to climb out of any open-topped vessel in which it is placed: in fact, it behaves as though its viscosity was zero, whence the appellation superfluidity. The frictionless flow of the electron gas in a superconducting metal, in many ways a similar phenomenon and equally unexpected at the time of its discovery, means that an electron current can flow forever around a closed loop of conductor without needing a driving voltage to maintain it; the technological implications of superconductivity, particularly if large current densities can be achieved at relatively high temperatures, have been well publicized in recent months.

In outline, the story of the discovery of superfluidity and superconductivity is a familiar one. It starts, in effect, with the liquefaction of helium — the last of the so-called permanent gases to succumb — by

Kamerlingh Onnes at Leiden in 1908. Once liquid helium was available for use as a cryogenic fluid, experiments went ahead on a wide range of materials at very low temperatures. In 1911, Onnes reported an exceptionally odd and, as it turned out, historic observation that the electrical resistance of mercury apparently fell abruptly to zero (that is, the



Kamerlingh Onnes — historic observation. (onset of superconductivity) as it was cooled through a transition temperature of about 4.2 K.

Although liquid helium continued to be used as a cryogen to cool a variety of experiments at Leiden and later at several other laboratories, and although many researchers were aware that it was an unusual liquid, its really remarkable property (of superfluidity) was not appreciated until surprisingly late, in 1938, with the famous Volume 141 of *Nature* in which workers in Cambridge, Moscow, Oxford and Kharkov reported almost simultaneously a whole range of phenomena leading in different ways to the same conclusion: that liquid helium below 2.17 K is a superfluid. The authors describe the history of these events in considerable detail,

mainly through reference to the literature, but also on the basis of their visits to Leiden and other centres of low-temperature research, and in the light of their discussions and correspondence with survivors from the latter part of the era in question, and with their collaborators and successors. They concentrate mainly on the period 1908–1956, but also provide valuable background on earlier years and an outline of developments after 1956.

The discovery of superconductivity certainly occurred “out of context”. It came while the quantum theory was in its infancy, before the development of quantum statistical mechanics, and it far preceded the advent of those theories of the solid state which were to be a prerequisite for its proper understanding. Superfluidity also arrived “out of context” but, coming more than a quarter of a century later, somewhat less so. The authors discuss in fascinating detail the twists and turns of the argument and the interplay between experiment and theory which led, in the end, to reasonably satisfactory understanding of the two phenomena by the mid-1950s. They could also have pointed out, however, that in the case of superconductivity, the resultant theory — the Bardeen–Cooper–Schrieffer (BCS) theory — was a good deal fuller, more fundamental and generally more satisfactory than that for helium (an uneasy co-existence of the early ideas of London, Tisza and Landau, together with the later unifying contributions of Feynman and others).

The approach taken by Gavroglu and Goudaroulis in analysing these events relies on their idea of “concepts out of context(s)” wherein a paradoxical situation is created by the initial attempts to describe a new phenomenon, precisely because it is incompatible with prevailing theory. The resolution of the paradox involves the development of a new theoretical framework, providing a context within which the phenomenon in question will be expected, rather than unexpected. Few scientists are likely to disagree with such an interpretation, which, stripped of the authors’ jargon, is a seemingly obvious one.

Gavroglu and Goudaroulis tell us a tale of considerable intrinsic interest involving technical ingenuity, astonishing discoveries, imagination, insight, bitter arguments between proponents of conflicting theories and, ultimately, triumph in terms of scientific understanding. The story is carefully told, with copious annotations and references to the original literature. There are some obvious lacunae, however, particularly in relation to historical perspectives on superfluid helium. For example, in the course of their description of the early controversy surrounding London’s seemingly outrageous suggestion that Bose–Einstein condensation (where, in



an ideal gas at a low enough temperature, particles 'condense' into the zero momentum ground state) might also occur in liquid helium, the authors do not mention that London was eventually proved correct by the neutron-scattering experiments of Eric Svensson and co-workers at Chalk River. Similarly, they provide an account of the Landau critical velocity paradox — Landau having predicted a critical velocity for the onset of frictional effects in superfluid helium that was several hundred times larger than the critical velocity actually observed in the early experiments — and of Feynman's resolution of the paradox in terms of dissipation through vortex production at lower velocities. But they forbear to mention that Landau was actually correct all along; the critical velocity for the onset of non-vortex dissipation, subsequently measured at Lancaster, was in excellent quantitative agreement with the original Landau prediction.

The saga is likely to be of interest to historians and philosophers of science. It will certainly provide fascinating reading for physicists, and in particular for all those involved in research at low temperatures. □

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## Greater clarity on crystals

Alan L. Mackay

**Introduction to Quasicrystals.** Edited by Marko V. Jarić. *Academic:1988. Pp.285. £31.50, \$50.*

MOST textbooks on crystallography and solid-state physics begin with the (still undoubted) proposition that, in an infinite crystallographic lattice, lattice points, all of which have identical surroundings, cannot have fivefold symmetry. Their authors also assume, and would like to prove, that the most stable state of an assembly of identical units is a crystal. They fail to prove this proposition rigorously, perhaps because it may not be true, and then continue with the physics of crystals.

Crystallographers have long been aware of the existence in crystals of molecules and clusters of icosahedral symmetry, and of the frequent incompatibility of the local symmetry of such groups with the limited point-site symmetries available in the 230 crystallographic space groups. Fivefold or icosahedral symmetry in extended crystals was universally assumed to be impossible. It was, therefore, a tremendous surprise when, in 1984, Dan Shechtman and his colleagues at the National Bureau of Standards in the United States reported that extended regions of a rapidly quenched alloy, of composition about Al<sub>63</sub>Mn, showed electron-diffraction patterns with sharp spots and with full icosahedral symmetry. Since the appearance of their paper, some 600 further papers on the new structures, now called 'quasi-crystals', have been published. With the present volume we now have the first book on the topic, presenting the material in more digestible form (a convenient basic compendium of papers, with commentary, had earlier been produced by Steinhardt and Ostlund).

There is nothing actually wrong with the crystallographic rules which we knew before 1984, but we have had to look much more closely at the gaps between them. Curiously, the phenomenon turns on unobserved phase angles. In X-ray diffraction from crystals, the Bragg's insight was to see that each spot in the diffraction pattern corresponded to a sinusoidal density wave of scattering matter and that the crystal could be considered as the linear superposition of these. The amplitudes of the waves are easily recorded but their phases are not. It is the art of X-ray crystal structure analysis to recover them.

The 230 space groups prescribe the symmetry relationships between the phases from the assumption of crystallicity. We are still free to make structures

with some particular symmetry for the amplitudes but no necessary symmetry for their phases, which may also vary from place to place in the material. People had assumed that any structure that gave diffraction patterns with sharp spots must be periodic. It has now been shown that materials with two incommensurate periodicities combined in the right way (by being a section through a higher-dimensional lattice), or being quasi-periodic in the strictly mathematical sense of the term, also show sharp spots.

It turns out that quasi-crystals exploit this unobservability of the phases. The diffraction effects may have icosahedral symmetry but that does not mean that there are fivefold axes in the structure. Since about 1974, the Penrose tiling has been known as a mathematical game and it was immediately seized upon as a patterning system which would produce diffraction phenomena like those observed. The Penrose tiling exemplified the extremely ingenious compromise found by nature between the demands of local icosahedral order and of long-range order.

Since 1984 the matter has become immensely more complicated. In the 1930s crystallographers discussed all kinds of structures between perfect crystals and glasses, and indeed the possibility of icosahedral textures was explicitly mentioned. But recently the initiative has passed to the solid-state physicists, who have attacked the area in massive strength.

In this volume, the first of a series entitled "Aperiodicity and Order", Marko Jarić has collected together six essays which lay out the new subject coherently. To start with, David and Clara Shoemaker describe the local icosahedral coordination often observed in metallic crystals, some of great complexity, which lays the foundation of local order — one of the consolations of ageing is that one can enjoy seeing their stereo-pairs without a stereoscope. Michael Widom looks at short- and long-range order more generally, introducing the intriguing relevance of four-dimensional polytopes as poly-tetrahedral structures with local icosahedral coordination, and discussing how they may be decurved back into the normal three-dimensional world in which the quasi-crystals must exist. Robert Schaefer and Leo Bendersky then provide a metallurgical setting for the occurrence of quasi-crystalline phases.

Per Bak and Alan Goldman begin on the new quasicrystallography, showing how structures with the necessary diffraction properties can be derived as projections of lattice structures in higher dimensional space. The phases with icosahedral diffraction symmetry can be derived as three-dimensional sections through a six-dimensional hypercubic lattice. Accordingly, diffraction spots can be assigned six-

integer indices with respect to this lattice; there is an analogy with the way in which ordinary hexagonal crystals are given four indices with respect to a four-dimensional lattice.

The remaining two chapters — "Stability and Deformation" by O. Biham, D. Mukamel and S. Shtrikman, and "Symmetry, Elasticity and Hydrodynamics in Quasiperiodic Structures" by T.C. Lubensky — take the reader into the field of solid-state physics rather than conventional crystallography. It is clear that crystallographers will have to absorb this new material. It might even be said that while they have been away capturing large areas of biology, the physicists have moved in and occupied the central camp of crystallographic theory. Crystallographers have become the victims of their own success (based on crystal structure analysis); phases, such as liquid crystals, which were somewhat disdained as being

inferior, have been taken over by solid-state physicists who have applied sophisticated theoretical analyses which crystallographers can hardly match.

Altogether, the quasicrystal episode has been of the greatest interest. The whole area of crystal symmetry — which is often quoted as being one of the most tightly sewn up with a beautiful and complete theory — has been broken open by an experimental observation, resulting in a mass of exciting new theories, many of which can be connected with actual materials. We can look forward to further books on the subject, where the new ideas will be laid out with increasing clarity and simplicity. The opening chapters of all volumes on crystallography and solid-state physics must henceforth begin differently. □

Alan L. Mackay is a Professor in the Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK.

print: "The work of the last ten years has emphasized the extreme difficulty of producing any really quantitative theory".

The appearance of Alan Cottrell's new book *Introduction to the Modern Theory of Metals* is indicative that the theoretical physicists have now put their house in order and can offer the metallurgist reliable quantitative insights into the world of metals at the atomic level. The magic touch, which he displayed in his earlier textbooks on metallurgy, is still very much in evidence as he describes the developments of the past 30 years that have placed the modern theory of metals on such a firm foundation. Coming from outside the field of electron theory himself, he offers a broad vision and a clarity of purpose.

The book begins with a discussion of the fundamental question 'What is a metal?'. The recent discovery of high-temperature superconductivity in ceramic oxides has driven home the fact to a wide audience that Wilson's original distinction between metals and insulators in terms of energy bands and Brillouin zones is fatally flawed. Cottrell stresses that physicists and chemists are now converging in their views on highly correlated systems — Mott's famous criterion for the metal-insulator transition being similar to a pre-quantum treatment of the problem as a polarization catastrophe by Herzfeld and Goldhammer, and Anderson's model of high-temperature superconductivity building on Pauling's ideas of the resonating valence bond. One-electron energy-band theory is, however, shown to be well justified for normal metals in which the electrons are less highly correlated. Excellent chapters introduce the ideas of quasiparticles, pseudopotentials and density functional theory which are at the heart of modern quantitative electron theory. Others discuss the cohesion and structure of simple metals, transition metals and their surfaces, and recent theories of alloy formation.

*Introduction to the Modern Theory of Metals* will be essential reading for students of metallurgy and solid-state physics. The mathematics is kept to a minimum within the main text, the more detailed working and theory being relegated to appendices, so that in Cottrell's hands even the mathematically weakest student should be able to follow the physical arguments. Research workers wishing to understand recent advances in the electron theory of metals will also find the book invaluable. With industrial laboratories the world over engaging electron theorists to help them in the search for new and better alloys, Raynor's optimism no longer appears to be so misplaced. The electron theory of metals has come of age. □

D. G. Pettifor is a Professor in the Department of Mathematics, Imperial College, Prince Consort Road, London SW7 2AZ, UK.

## Electronified

D. G. Pettifor

**Introduction to the Modern Theory of Metals.** By Alan Cottrell. *Institute of Metals, 1 Carlton House Terrace, London SW1Y 5DB/Old Post Road, Brookfield, Vermont 05036:1989. Pp.260. £35, \$73.50.\**

THE classic textbooks by Hume-Rothery and Raynor on the electron theory of metals were published soon after the end of the Second World War. They were imbued with the optimism, prevalent at that time amongst scientists, that science could win the peace just as it had helped win the war. In response to the query why metallurgists should know about quantum theory, Raynor writes in his 1947 preface: "If the metallurgists of [Britain] do not learn about electron theories, they may discover, too late, that industry in other countries has benefited from knowledge of modern theories and is developing new alloys".

This belief in the power of electron theory to place the metallurgical industry on sound scientific foundations did not seem misplaced. Hume-Rothery's pioneering work on the solubility limits and crystal structure of copper, silver and gold alloys had been given a simple theoretical

explanation by Jones in 1934. Experimentally, the maximum solubility of polyvalent elements such as zinc, aluminium or tin in the monovalent noble metals was found to occur at an average number of valence electrons per atom of approximately 1.4 (assuming a valence of 1, 2, 3 and 4 for copper, zinc, aluminium and tin respectively). Jones pointed out that this was very close to the number of electrons per atom which is required for the spherical free-electron Fermi surface to make contact with the Brillouin zone boundary corresponding to the face-centred cubic lattice of the noble metals. Further solute atoms would push electrons into the high-energy states across the gap at the zone boundary. This is energetically unfavourable and Jones predicted the face-centred cubic lattice would undergo a transformation to the body-centred cubic lattice, as is observed experimentally.

Alas, this optimism in the usefulness of modern theory for alloy development was dashed ten years later when, in 1957, Pippard discovered that the Fermi surface had already made contact with the Brillouin zone boundary in elemental copper, even though the electron per atom ratio was only one. The approximation of treating copper's valence electrons as a gas of free electrons which are only weakly perturbed by the ionic lattice — a problem which theorists could solve — was simply too crude. In practice, the full 3d electronic shell in copper is not inert but hybridizes strongly with the valence sp band, thereby giving copper its characteristic colour and high cohesive energy. At the time, however, controversy surrounded how best to treat the valence d electrons in transition and noble metals — whether to regard them as localized on their parent atoms or itinerant. As Hume-Rothery wrote in the preface to the 1962 fourth (revised) re-

\* To coincide with the publication of Cottrell's book, The Institute of Metals has reprinted three titles on the same or related topics — *Atomic Theory for Students of Metallurgy*. By W. Hume-Rothery and B. R. Coles. Pp.428. Pbk £18, \$37.80. *An Introduction to the Electron Theory of Metals*. By G. V. Raynor. Pp.98. Pbk £10, \$21. *The Structure of Metals and Alloys*. By R. E. Smallman, W. Hume-Rothery and C. W. Haworth. Pp.407. Pbk £18, \$37.80.



## Mother love

Leonard Krishtalka

**Digging Dinosaurs.** By John R. Horner and James Gorman. Workman Publishing, New York: 1988. Pp.210. \$17.95.

A HUNDRED years ago in the American West, a 'bone pilgrim' was a cowboy who collected dead animal bones and sent them east for processing into artificial manure. Roaming the West at the same time was a second, lesser known, breed of bone pilgrim, one who collected fossil animal bones and sent them east for processing into scientific manuscripts. Jacob Wortman, Charles Sternberg, William Reed, John Bell Hatcher and others were bone pilgrims of this sort for Pittsburgh's Carnegie Museum, New York's American Museum of Natural History, Yale's Peabody Museum and Princeton University. Today they would be called 'field palaeontologists'.

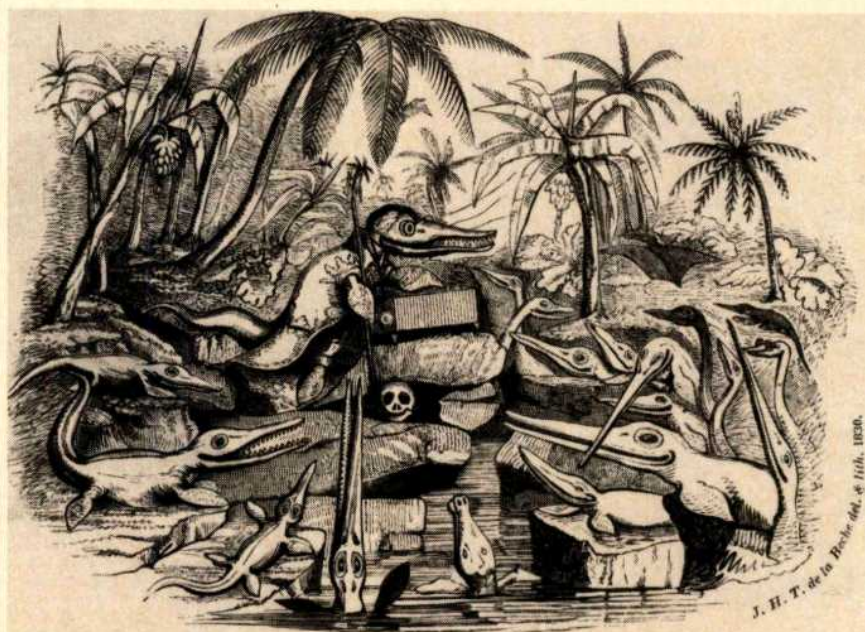
John Horner, curator of palaeontology at the Museum of the Rockies in Bozeman, Montana, would have made a fine bone pilgrim in the Old West because he is one of the finest field palaeontologists in the New West. In August 1978 Horner, then a preparator of fossils at Princeton, and his friend Bob Makela, a high-school science teacher in Montana, followed the owners of a local rock shop up a windswept knob of red and green mudstone on the plains near Choteau, Montana. Back at the rock shop lay bits of a thigh bone, a rib and a jaw of a baby hadrosaur (a duck-billed dinosaur) that the owners had found on the surface.

Horner and Makela combed the knob, and in the scree found the remains of two more baby hadrosaurs. The bones were concentrated in a bowl-shaped mass of green mudstone, a nest scooped out by the hadrosaur mother 80 million years ago. The dinosaur was a new genus, later named *Maiasaura*, 'good mother lizard'. Horner and his colleagues spent the next six years in Montana exhuming the ancient life of *Maiasaura* from the knob and surrounding rock — 14 nests, 42 eggs, three nesting grounds, 31 babies and a gigantic herd of 10,000 individuals. The excavations also uncovered the extraordinary record — 25 skeletons, 14 clutches of eggs and 19 eggs with embryonic skeletons — of a second dinosaur, a new genus of hypsilophodontid.

In a field beset by hyperbole, it is fair to say that Horner's discoveries are unparalleled. Two square miles of late Cretaceous rocks reveal geological moments when female maiasaurs and hypsilophodontids gathered in birdlike flocks on communal nesting grounds and laid their eggs. On hatching, the young maiasaurs (which were apparently altricial) stayed in their

## AWFUL CHANGES

MAN FOUND ONLY IN A FOSSIL STATE — REAPPEARANCE OF ICHTHYOSAURI.



A Lecture — "You will at once perceive," continued Professor Ichthyosaurus, "that the skull before us belonged to some of the lower order of animals; the teeth are very insignificant, the power of the jaws trifling, and altogether it seems wonderful how the creatures could have procured food."

The cartoon, from Francis T. Buckland's *Curiosities of Natural History* (1859), is reproduced in *Dinosaur Plots and Other Intrigues in Natural History* by Leonard Krishtalka, published by William Morrow, which will be reviewed in *Nature's Spring Books* issue next week.

nests and were fed and cared for by their good mother lizard; the hypsilophodontid hatchlings, comparatively more mature, left the nests immediately after birth. Both species, like birds, grew quickly and were warmblooded. The maiasaurs, like modern wildebeest, travelled in herds several thousand strong.

*Digging Dinosaurs*, which is co-written with James Gorman, is Horner's personal account of this revolutionary dinosaur diorama and the good fortune, perseverance and palaeontological detective work behind its discovery and reconstruction. Horner is folksy. He toasts the co-evolutionary ties of bonehunters and beer, using the tab of a Rainier beer can for scale in an illustration of baby maiasaur bones. Not much has changed in a hundred years of bonehunting: the field pictures are no longer black and white, the field crews no longer wear black suits and bow-ties in the quarry, and mule teams no longer haul out the dinosaur blocks. But that is about all. What has changed is the picture we have of the dinosaurs themselves, no longer cloddish and dimwitted, but hot, nimble, social and caring.

The book captures the romance, realism and scientific implications of Horner's field work and astonishing discoveries. The friendly, unassuming tone is tailored

for a general audience; those hungry for the straight, academic treatment of the evidence can refer to his papers in the scientific journals. Once in a while Horner's folksy science is, as they say in the West, bodaciously wrong — life evolved at least 3.5 not 2 billion years ago (pp. 38–39), and Darwin never called natural selection 'survival of the fittest' (p.66), Herbert Spencer did. Some critics have already demanded more evidence for maternal care among maiasaurs. Others question Horner's proposition that volcanic gassing and catastrophic flooding turned a herd of 10,000 of them into a graveyard of dismembered, shattered skeletons.

But these are the rough and tumble issues in *Digging Dinosaurs*. When it comes to dinosaurs, the palaeontologist's mind until recently was a prisoner of conventional attitudes that kept the beasts stiff and cold. As Horner says, "palaeontology can grow sleepy and accumulate dust like museum cellars" were it not for challenges to the prevailing wisdom. Those challenges have wrought a dinosaur renaissance during the past two decades, a new manifesto for the Mesozoic. □

Leonard Krishtalka is Curator in the Section of Vertebrate Paleontology, Carnegie Museum of Natural History, 4400 Forbes Avenue, Pittsburgh, Pennsylvania 15213, USA.



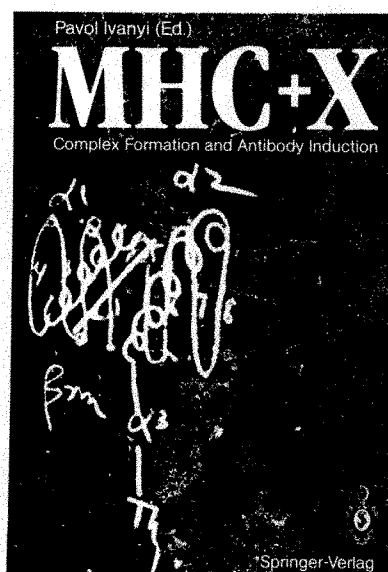
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K. Rother, University of Heidelberg; G. O. Till, University of Michigan (Eds.)

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# Climate change in the circum-North Atlantic region during the last deglaciation

Jonathan T. Overpeck\*, Larry C. Peterson†, Nilva Kipp‡, John Imbrie‡ & David Rind§

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A survey of new and published palaeoclimate data indicates that both the high- and low-latitude North Atlantic regions were characterized by at least three synchronous periods of abrupt climate change during the last glacial-to-interglacial transition. Climate model results suggest that changes in the melting history of the Laurentide Ice Sheet may explain much of this nonlinear response of the climate system to astronomical (Milankovitch) forcing.

ASTRONOMICAL or Milankovitch forcing has become established as the primary 'pacemaker' of the ice ages, accounting for much of the total observed climatic variance of the late Quaternary<sup>1,2</sup>. Most climatic variance at frequencies lower than 1 cycle per 19,000 years is related to astronomical forcing, and even abrupt glacial terminations can be partially attributed to such forcing<sup>3</sup>. A closer look at the last glacial-interglacial transition, however, reveals a sequence of climate change that cannot be explained as a simple linear response to astronomical forcing. Marine and terrestrial records of environmental change in the high-latitude North Atlantic, Greenland and Europe reveal a period of rapid climatic warming at ~13–12.6 kyr BP, followed by an abrupt reversal towards colder glacial conditions at ~11 kyr, and a second rapid warming to interglacial conditions at ~10 kyr (refs 4–7). The abrupt 13-kyr warming and the 'Younger Dryas' cold event between 11 and 10 kyr were apparently more pronounced over Greenland and Europe than over North America, an observation that has led workers to surmise that changes in the heat budget of the high-latitude North-Atlantic produced the changes over land<sup>5–9</sup>. Ruddiman<sup>4</sup> proposed that a reduction in ice-sheet height over North America could have rapidly reduced the amount of cold air advected

over the North Atlantic, thus allowing the North Atlantic and downstream Europe to warm abruptly at ~13 kyr. Ruddiman<sup>4</sup> and Broecker *et al.*<sup>5</sup> expanded on earlier work<sup>10–13</sup> to suggest that major diversions of Laurentide Ice Sheet meltwater between the Mississippi and St Lawrence rivers may have caused the North Atlantic, Greenland and Europe to cool at the beginning of the Younger Dryas period (~11 kyr) and then warm abruptly at the end of the Younger Dryas period (~10 kyr). General Circulation Model (GCM) results support the dominant role of North Atlantic sea surface temperature (SST) fluctuations in forcing high-latitude climate change<sup>6,9</sup>.

Here we try to place the observed high-latitude pattern of climate change into a more global perspective. First, we examine new and published data from the Caribbean and Gulf of Mexico region that also point to systematic patterns of abrupt change at ~13–12.6, 11 and 10 kyr. We then examine new and published climate model results which suggest that much of the low-latitude pattern of change can be linked to variations in meltwater discharge down the Mississippi and St Lawrence rivers, and the sea surface temperatures of the high-latitude North Atlantic and Gulf of Mexico. Our results lead us to speculate that rapid climate events over Africa may also have been related to these same variations. We conclude with the hypothesis that the climate system translated the relatively gradual astronomical forcing, characteristic of the past 18 kyr, into an inter-related sequence of abrupt climate events over an extensive area of the globe.

## Caribbean and Gulf of Mexico regions

In this section, we look at the patterns and sequence of climate change which occurred around the Caribbean and Gulf of Mexico during the last deglaciation. In particular, we focus our initial attention on data derived from an investigation of sediments from the Cariaco Basin (10°40' N, 65° W), a small (160 × 40 km), deep (1,400 m) anoxic marine basin aligned along the northern continental margin of Venezuela. This margin is

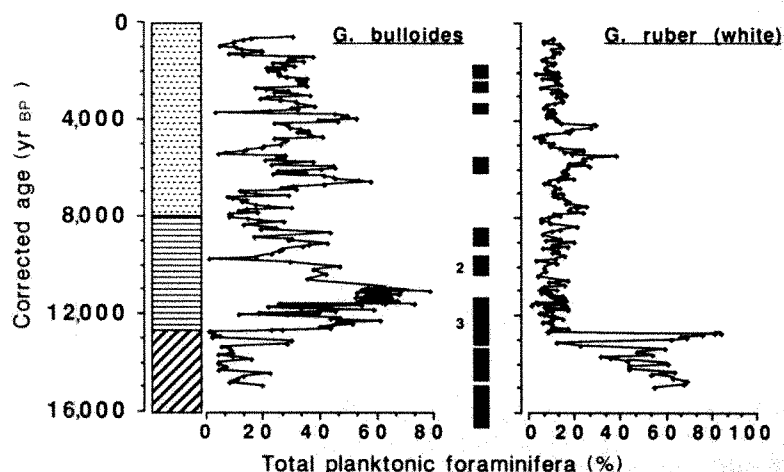


FIG. 1 Time series of *G. bulloides* and *G. ruber* relative abundance for core V12-99 from the Cariaco Basin. Also shown are core lithology and the distribution of radiocarbon-dated intervals which were biostratigraphically correlated from the closely matching, but less continuous, core V12-104. This series of dates did not include any reversals. All dates were adjusted by 400 years to correct for the difference between surface water and atmosphere radiocarbon content. The V12-104 record is less continuous due to the presence of turbidites that were not present in V12-99. ■, radiocarbon-dated intervals (2, 3 = number of dates); Sediment type: □, weakly laminated calcareous clays; ■, strongly laminated calcareous clays; ▨, weakly bioturbated calcareous clays.

currently characterized by Ekman-induced coastal upwelling, with cold SSTs found during the dry winter/spring season of greatest along-shore wind stress, and warmer SSTs during the less windy wet summer season<sup>14,15</sup>. Plankton tow data from the Cariaco Basin indicate that seasonally high abundances of phytoplankton (mainly diatoms) and the planktonic foraminifer *Globigerina bulloides* dominate the water-column assemblage during the upwelling season, whereas *Globigerinoides ruber* is abundant during the non-upwelling season<sup>16</sup>.

The strong seasonality in the wind-induced coastal upwelling is reflected in the faunal and lithological character of the laminated sediments which accumulate rapidly (20–100 cm kyr<sup>-1</sup>) beneath anoxic waters in many portions of the Cariaco Basin. Piston cores V12-99 (1,005 m water depth) and V12-104 (466 m) were sampled on average every 3 and 1 cm, respectively, for a complete foraminiferal census. Age control is provided by twelve radiocarbon dates on bulk carbonate (Fig. 1). We subtracted 400 years from each date to account for the difference in radiocarbon content between the surface waters and atmosphere<sup>7</sup>. The two piston cores, separated by over 50 km, yielded nearly identical time series of foraminiferal abundance, indicating that our faunal records are probably representative of the basin as a whole. We focus here on the abundance record of *G. bulloides* in V12-99 as a proxy record of upwelling, and hence trade-wind intensity (Fig. 1). We will elaborate further on the very detailed Cariaco Basin record elsewhere. Like the high-latitude records of climatic change, the Cariaco Basin record shows evidence of rapid change at ~13–12.6, 11 and 10 kyr.

The presence of weakly bioturbated sediments, the high abundance of *G. ruber*, and the low abundance of *G. bulloides*

near the base of the core (Fig. 1) suggest that lowered sea level may have isolated the Cariaco Basin sufficiently to prevent active Ekman-pumping during the last glacial maximum. At 12.6 kyr, both cores record a significant increase in sedimentation rate, an abrupt change from bioturbated to distinctly laminated anoxic sediments (traceable over the entire basin), and a sudden shift in foraminiferal assemblages from a non-upwelling fauna to samples dominated by *G. bulloides*. High concentrations of *G. bulloides* during the period 12.6–10.8 kyr (>2,000 individuals g<sup>-1</sup> compared to a core mean of <500) suggest that the high sedimentation rates observed throughout this period (>80 cm kyr<sup>-1</sup> compared to the core mean of <40) were related to intense upwelling and high productivity of *G. bulloides*. The abundance of *G. bulloides* drops abruptly after 10.8 kyr, but remains at levels above the Holocene mean. This change suggests that upwelling and trade-wind intensities, although diminished, were still high during the period 10.8–10 kyr. A further decline in the abundance of *G. bulloides* after 10 kyr suggests that the Holocene mode of moderate (although still highly variable) upwelling began at this time.

The Cariaco Basin upwelling record provides evidence for rather abrupt changes in trade-wind strength at ~12.6, 10.8 and 10 kyr, with the strongest trade winds over the southern Caribbean found between 12.6 and 10.8 kyr. Independent palaeoclimate data from the region support the abrupt nature of change at these times. In the lowlands adjacent to the Caribbean, pollen and lake-level records indicate that glacial (18 kyr) aridity persisted until shortly after 11 kyr, when moist conditions suddenly prevailed<sup>17–19</sup>. To the north of the Gulf of Mexico and Caribbean region, the deglacial sequence of climate change is less clear.

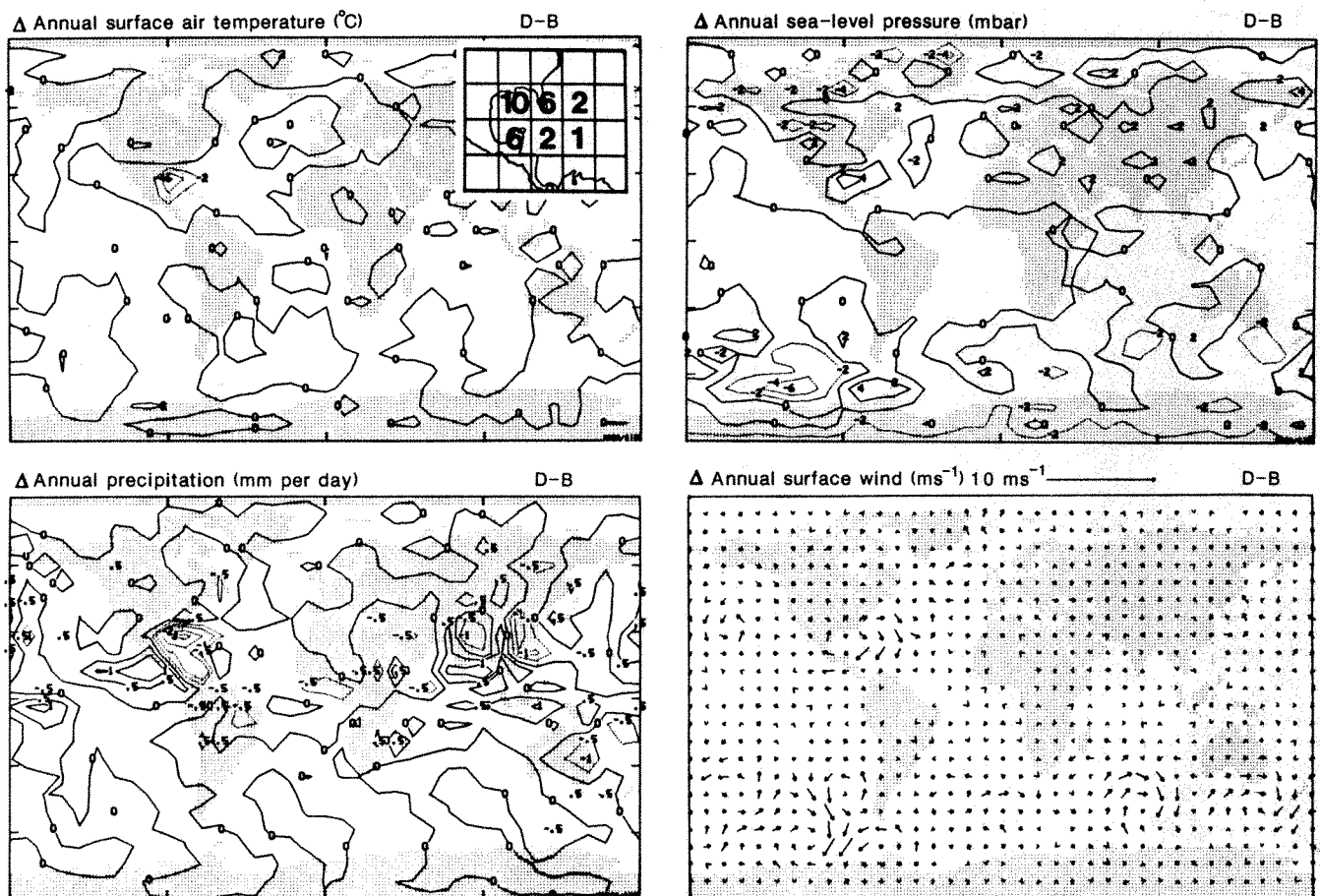


FIG. 2 Simulated mean-annual differences between experiment D and B. Inset shows the magnitude of the negative (°C) SST anomalies (relative to

the present-day) that were prescribed for six model grid points in experiment D (see Table 1 and text for more details).



Water levels in low-elevation lakes throughout the southeast US Coastal Plain were low or lakes were dry from before 18 kyr until after 15 kyr, when lake levels rose in response either to increased precipitation, rising sea level, or both<sup>20</sup>. A similarly equivocal record of past hydrological change in this region is the poorly dated record of fluvial changes which seems to indicate moist conditions until 13 kyr (refs 21, 22). Pollen records from the southern Coastal Plain<sup>20,23–27</sup> show abrupt change at 13 and 11 kyr. Increased amounts of herb pollen and decreased abundances of *Carya* pollen may argue for aridity between 13 and 11 kyr in Florida<sup>20,23,24</sup>, just as increased amounts of *Fagus* and *Liquidambar* pollen may have signalled the onset of wetter conditions after 11 kyr further north<sup>25–27</sup>. More data are clearly required, however, to refine this regional picture.

### Observed versus simulated palaeoclimatic change

The apparent synchronicity between events in the Cariaco Basin record, in other records from the Caribbean and Gulf of Mexico region, and in records from the high-latitude North Atlantic suggest a common forcing mechanism during deglaciation. In particular, the evidence for stronger trade winds between 12.6 and 10.8 kyr in the western North Atlantic and for Gulf/Caribbean aridity until ~11 kyr have led us to examine the effects that a cool Gulf of Mexico may have had on the climate system. Geological data from North America and stable isotope data from Gulf sediments suggest that the flow of cold, fresh Laurentide Ice Sheet meltwater down the Mississippi River and into the Gulf of Mexico began at ~15–14 kyr, waned slightly from ~14–13 kyr, and peaked between 13 and 11 kyr before being diverted down the St Lawrence River into the high-latitude North Atlantic<sup>5,9–12</sup>. During the interval of maximum discharge into the Gulf, which coincides closely with the timing of events described above, the volume of Mississippi outflow must have been immense (5–11 times the present annual discharge, but mostly confined to the spring–summer meltwater season<sup>13,28</sup>), apparently included seasonal ice-rafted detritus (R. Saucier, personal communication), and was sufficient to affect sea surface salinities well out into the western North Atlantic<sup>13,29</sup>. Unlike the role of salinity, little attention has been paid to the possible consequences of meltwater-induced temperature change in the Gulf of Mexico. Independent data<sup>30,31</sup> suggest that the open south-west Caribbean did not warm from glacial values until ~11 kyr, and it seems likely that the more northerly Caribbean and Gulf of Mexico regions may have been cooled even further by the large influx of glacial meltwater. We suggest that cooler Gulf SSTs during peak meltwater discharge could provide a mechanism for explaining observed events if the cooling was sufficient to produce a high regional surface-pressure anomaly, which would in turn strengthen western Atlantic trade winds and suppress precipitation.

To test this hypothesis, we compared the results from four GCM ( $8^\circ \times 10^\circ$  latitude by longitude grid size) experiments (for details, see Table 1) using the Goddard Institute for Space Studies model<sup>6,32</sup>. Experiment A represents our model simulation of the current (warm) climate. The results of the four-year experiments, B and C, were compared to test the sensitivity of the global climate system to the hypothesized Younger Dryas (~11–10 kyr) reduction in North Atlantic SSTs<sup>6</sup>. We also ran a two-year experiment (D) to test the sensitivity of the climate system to cooled Gulf of Mexico SSTs during the period ~13–11 kyr. Experiment D was identical to B, except that model grid points which included the Gulf of Mexico and adjacent water were cooled by ~6 °C on average (Fig. 2). Unfortunately, the palaeoceanographic record provides no direct evidence for cooling of the Gulf by meltwater. However, the oxygen isotope record of meltwater<sup>10,11,13,29</sup> is useless in this regard because of the predominance of salinity effects, and existing microfossil-based studies of Gulf SSTs<sup>33,34</sup> have sample spacings that may preclude recognition of such a brief (2-kyr) event. In the absence of data to the contrary, we selected an average cooling of 6 °C

based on estimates of meltwater volume, on the observation that present-day Mississippi River water is up to 10 °C colder than Gulf surface waters<sup>28,35</sup>, and on the observation that downstream Caribbean SSTs were probably already depressed by 2–3 °C before they warmed at 11 kyr to Holocene levels<sup>30,31</sup>. Independently, Oglesby *et al.*<sup>28</sup> have arrived at a similar estimate and, more importantly, have performed sensitivity tests which suggest that an even smaller cooling could still have forced the same patterns of change around the Gulf that we have forced with our SST anomaly.

Our simulated cooling of the Gulf of Mexico (D minus B) produced lower surface air temperatures, higher sea-level pressure, a large negative precipitation anomaly, and a more vigorous anticyclonic surface air circulation over the Gulf of Mexico and Caribbean region (Fig. 2). Each of these changes is statistically significant (greater than several standard deviations of the inter-annual changes found in experiment B) and agrees with the palaeoclimatic data described in the previous section. The simulated difference between experiments D and A indicate that a cool Gulf of Mexico between ~13 and 11 kyr is needed to match the palaeoclimatic evidence for greater than present aridity around the Gulf and Caribbean region, and more intense trade winds in the western North Atlantic. Averaged over the annual cycle, the cooling of the Gulf produced no significant temperature anomalies outside the Gulf/Caribbean region. In our experiment, the well-developed higher pressure and cooler temperatures in the Gulf region helped divert storm tracks northward, resulting in both lower pressure and a small band of increased precipitation north of the Gulf Coastal Plain. This pattern agrees with the observation that aridity may have been restricted to Florida and Georgia between ~13 to 11 kyr. Slightly higher precipitation values, probably indicative of increased Hadley circulation, were also simulated equatorward of the Gulf of Mexico over the northern Amazon Basin and central Andes. These changes were not as significant (less than three standard deviations) as those simulated around the Gulf of Mexico and Caribbean region, but they do appear to agree with the sparse palaeoclimate data from South America<sup>36,37</sup>.

Rind *et al.*<sup>6</sup> compared C and B to demonstrate the importance of high-latitude North Atlantic SSTs in forcing the observed climate changes over North America, Greenland and Europe during the last glacial–interglacial transition. Our comparisons of D with B (Fig. 2) and C with D (Fig. 3) support this conclusion. On a yearly average basis, the observed high-latitude variations in both precipitation and temperature appear to be explained by the observed changes in high-latitude SSTs: rapid warming at ~13 kyr, rapid cooling at ~11 kyr (the onset of the Younger Dryas) and rapid warming again at ~10 kyr (the end of the Younger Dryas). At lower latitudes, the warming of the Gulf at ~11 kyr can explain the wetter conditions and slight wind relaxation observed at this time in the western North Atlantic/Gulf/Caribbean region (Fig. 3). The magnitude of the

TABLE 1 Summary of General Circulation Model experiments

Designation	Description
A	current climate <sup>32</sup>
B	11 kyr orbital parameters <sup>50</sup> 11 kyr land ice <sup>49</sup>
C	current sea surface temperatures 11 kyr orbital parameters <sup>50</sup> 11 kyr land ice <sup>49</sup>
D	current sea surface temperatures, except in the North Atlantic north of 25°N where they were cooled to their ice age values (18 kyr) <sup>51</sup> 11 kyr orbital parameters <sup>50</sup> 11 kyr land ice <sup>49</sup> current sea surface temperatures, except in the Gulf of Mexico region where they were cooled on average 6 °C (see Fig. 2)

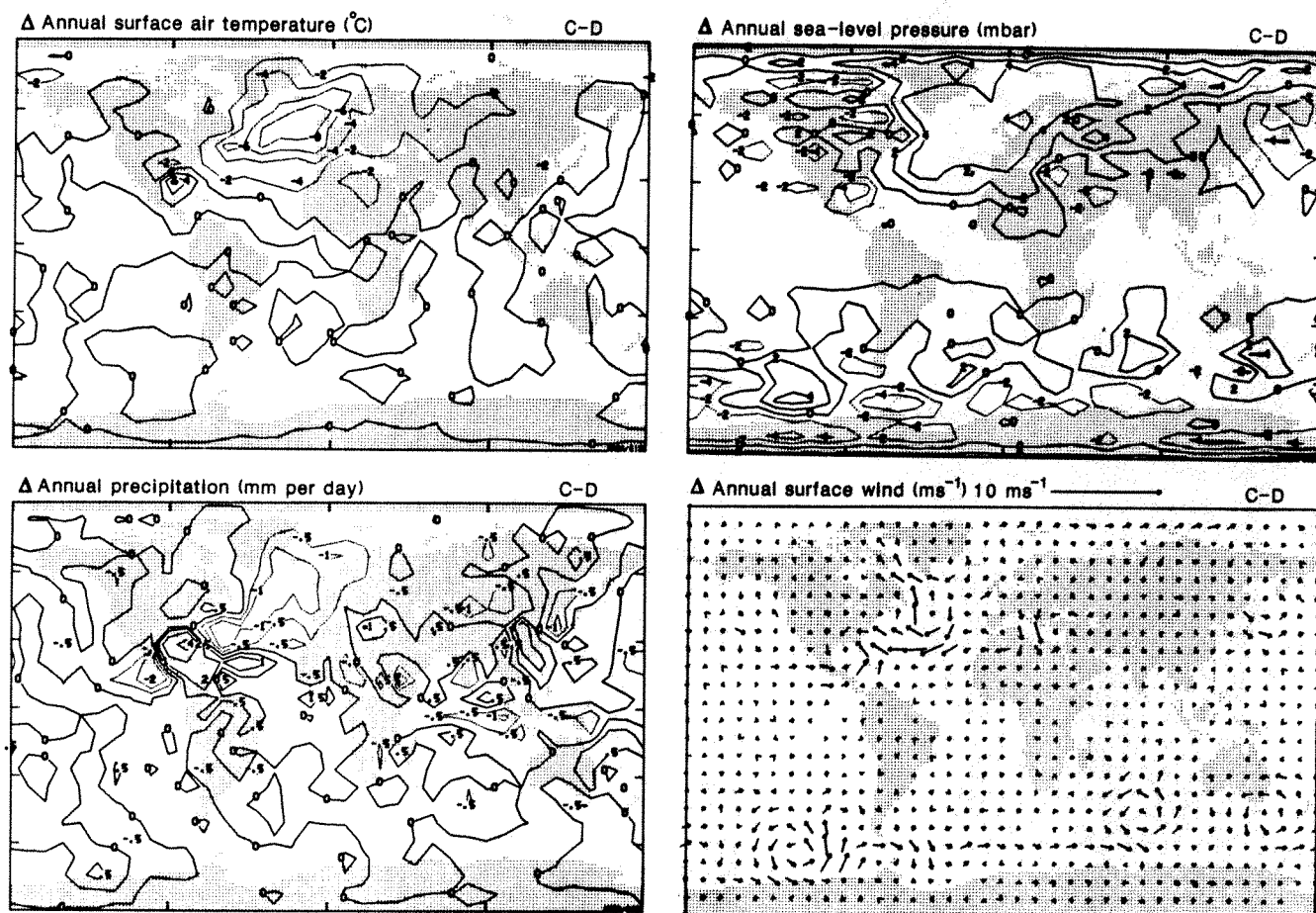


FIG. 3 Simulated mean-annual differences between experiment C and D (see Table 1 and text for more details).

Gulf/Caribbean aridity probably would have been enhanced between 13 and 11 kyr had we prescribed colder SSTs along the northern South American margin in accordance with the Cariaco Basin upwelling record.

Our simulations suggest that the observed pattern of climate change over most of the circum-North Atlantic region can be explained by the proposed effects of cold Laurentide Ice Sheet meltwater on SSTs of the high-latitude North Atlantic and Gulf of Mexico. Our model results may not, however, provide a complete physical explanation for the sequence of change observed over North Africa, and, in particular, for the arid event that apparently was synchronous with the Younger Dryas in Europe ( $\sim 11$ –10 kyr, refs 38–46). The increased surface pressure and stronger easterly surface winds in experiment C relative to D (Fig. 3) support the idea<sup>39,44</sup> that a colder North Atlantic could have stabilized the lower atmosphere and helped block the advection of moisture over North Africa. However, the simulated precipitation changes between the two experiments are somewhat ambiguous (Fig. 3). Additional experiments are needed to examine the significance of the precipitation anomalies in East and North Africa. It is also necessary to test the possibility that abrupt changes of high-latitude North Atlantic SSTs were associated with other rapid changes that could have contributed to the observed changes over North Africa. Higher atmospheric aerosols<sup>47</sup>, changes in vegetation, a weaker monsoon, and lower-latitude Atlantic SST cooling<sup>48</sup> may all have helped to make North Africa more arid before  $\sim 13$ –12.6 kyr, and between 11 and 10 kyr.

### Conclusion

Our postulated synchronicity of rapid climate events at  $\sim 13$ –12.6, 11 and 10 kyr around the circum-North Atlantic must be tested

by means of the acquisition of additional well-dated, high-resolution palaeoclimate records. In most cases, we were forced to use dates with an uncertainty of  $\sim 5$ –10% to infer synchronicity. In addition, further efforts to evaluate the palaeotemperature record from the Gulf of Mexico are clearly warranted. These palaeoclimatic studies will have to be augmented by additional climate model experiments to understand the sensitivity of the global climate system to deglacial changes in boundary conditions. Our present simulations, however, support a scenario through which the climate system (via the melting history of the Laurentide Ice Sheet) could have transformed relatively gradual and monotonic astronomical forcing into a series of abrupt, non-monotonic climate changes over a large part of the globe.

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# Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor

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Cloning and sequencing of the complementary DNA for platelet-derived endothelial cell growth factor indicates that it is a novel factor distinct from previously characterized proteins. The factor, a protein with a relative molecular mass of about 45,000, stimulates endothelial cell growth and chemotaxis *in vitro* and angiogenesis *in vivo*.

PLATELET-DERIVED endothelial cell growth factor (PD-ECGF) is an endothelial cell mitogen of relative molecular mass ( $M_r$ ) ~45,000 (45K) purified to homogeneity from human platelets<sup>1,2</sup>. By contrast with other endothelial mitogens of the fibroblast growth factor (FGF) family (reviewed in refs 3 and 4), PD-ECGF, which seems to be the only endothelial cell growth factor in human platelets<sup>2</sup>, does not bind to heparin and does not stimulate the proliferation of fibroblasts<sup>1</sup>. We describe here the protein sequencing, cDNA cloning and expression of functionally active PD-ECGF. In addition, we show that PD-ECGF has chemotactic activity for endothelial cells *in vitro* and angiogenic activity *in vivo*.

## Sequence of PD-ECGF

Information about the protein sequence of PD-ECGF was obtained by the N-terminal sequencing of intact PD-ECGF, and by analysing the fragments obtained after digestion with trypsin, staphylococcal V8 protease, or CNBr (Fig. 2).

To localize a source of production of PD-ECGF, a specific

antisera against PD-ECGF (ref. 2) was used to screen cell lines and tissues by immunoblotting. A strong 45K band was found when extract from a term placenta was analysed (data now shown). A cDNA library was therefore constructed in  $\lambda$ gt10 using poly(A)<sup>+</sup> RNA from human placenta, and screened with oligonucleotide probes constructed using the information from the protein sequencing; the screening of  $3 \times 10^5$  clones yielded three positive clones.

Nucleotide sequencing of the 1.8-kilobase (kb) insert of one of the clones (APL8) revealed a short GC-rich 5' untranslated region, an open reading frame predicting the translation of a 482-residue protein ( $M_r$  49,972), and a short 3' untranslated sequence containing a poly(A)<sup>+</sup> tail (Fig. 1). The translation is probably initiated by the ATG start codon at nucleotides 124-126, as the surrounding nucleotide sequence follows the rules for translation initiation<sup>3</sup>, whereas the sequence surrounding ATG at nucleotides 136-138 does not. Furthermore, there are no other ATG codons between an in-frame stop codon at nucleotides 28-30 and the nucleotides coding for the N-terminus of intact PD-ECGF (Fig. 2). A stop codon (TAA) is present at nucleotides 1,570-1,572, and is part of the polyadenylation signal (nucleotides 1,568-1,573). Fourteen nucleotides downstream of this signal, a long stretch of poly(A)<sup>+</sup> was found. A similar overlap of the stop codon and the polyadenylation signal has been reported for human choriongonadotropin B-chain<sup>6</sup>.

Of the 482 amino-acids of PD-ECGF deduced from the cDNA clone, 389 were identified by amino-acid sequencing (Fig. 2). The N-terminal sequence of PD-ECGF starts 10 amino acids downstream of the proposed translation initiation site, indicating that the molecule undergoes a limited proteolytic processing after synthesis. The C-terminal amino acids predicted from the

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cDNA sequence, except the last four, were identified by amino-acid sequencing. It is not known whether PD-ECGF undergoes proteolytic processing in the C-terminus, if so, a maximum of four amino acids are removed. The  $M_r$  of the mature protein would thus be 48.6–49K, in reasonable agreement with the estimate of 45K obtained from SDS-gel electrophoresis of pure PD-ECGF<sup>1,2</sup>.

The predicted amino-acid sequence from the cDNA clone matched the previously obtained amino-acid sequence except

at position 471, where the nucleotide sequence predicts Leu, whereas Ser was found in both of two different peptides obtained from this region (Fig. 2). It is possible that polymorphism occurs at this position.

Matching the sequence of PD-ECGF with those of the PIR, EMBL and Genbank databases (releases 17, 14 and 56, respectively) revealed no striking similarities to other proteins. Short internal repeats in the sequence were noted (overlined in Fig. 2). One important feature of the sequence is the lack of a

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1 GGGCAGTGGG CCGCTGTGGC CGAACCTGGA ACCCTACGGT CCGCAGCCGC GGGGAGGGCC
61 GGGTACCTGG CCGTGGGATCC GGAGCAAGCC GGGCAGGGCA GGGCCCTAAG CAGGCGCCGA
121 GCG ATG GCA GCG TTG ATG ACC CCG GGA ACC GCG GCC CCA CCC GCG CCG GGT GAG TTC TCC
1 Met Ala Ala Leu Met Thr Pro Gly Thr Gly Ala Pro Pro Ala Pro Gly Asp Phe Ser
20 Gly Gly Ser Gln Gly Leu Pro Asp Pro Ser Pro Glu Pro Lys Gln Leu Pro Glu Leu
181 GCG GAA GGG AGC CAG GGA CTT CCC GAG CCG TCG CCA GAG CCC AAG CAG CTC CCG GAG CTG
20 Gly Gly Ser Gln Gly Leu Pro Asp Pro Ser Pro Glu Pro Lys Gln Leu Pro Glu Leu
241 ATC CGC ATG AAG CGA GAC GAG GCG CCG CTG AGC GAA GCG CAC ATC AGG GCG TTC GTG GCC
40 Ile Arg Met Lys Arg Asp Gly Gly Arg Leu Ser Glu Ala Asp Ile Arg Gly Phe Val Ala
301 GCT GTG GTG AAT GGG AGC GCG CAG GCG CCA CAG ATC GGG GCC ATG CTG ATG GCG ATC CGA
60 Ala Val Val Asn Gly Ser Ala Gln Gly Ala Gln Ile Gly Ala Met Leu Met Ala Ile Arg
140 Val Pro Met Ile Ser Gly Arg Gly Leu Gly Thr Ser Val Leu Thr Gln Ala Leu Ala Gln Ser Gly
80 Leu Arg Gly Met Asp Leu Glu Thr Ser Val Leu Thr Gln Ala Leu Ala Gln Ser Gly
421 CAG CAG CTG GAG TGG CCA GAG GCG TGG GCG CAG CAG CTT CTG GAG AAG CAT TCC ACA GGG
100 Gln Gln Leu Glu Trp Pro Glu Ala Trp Arg Gln Gln Leu Val Asp Lys His Ser Thr Gly
481 GGT GTG GGT CAG AAG GTC AGC CTG GTC CTC GCA CCT GCC CTG GCG GCA TGT GCG TGC AAG
120 Gly Val Gly Asp Lys Val Ser Leu Val Leu Ala Pro Ala Leu Ala Cys Gly Cys Lys
541 GTG CCA ATG ATC AGC GGA CGT GGT CTG GGG CAC ACA GGA GGC ACC TTG GAT AAG CTG GAG
140 Val Pro Met Ile Ser Gly Arg Gly Leu Gly Thr Gly Gly Thr Gly Thr Gly Thr Gly Thr Gly
601 TCT ATT CCG GGA TTC AAT GTC ATC CAG AGC CCA GAG CAG ATG CAA GTG CTG GAG CAG
160 Ser Ile Pro Gly Phe Asn Val Ile Gln Ser Pro Glu Gln Met Gln Val Leu Leu Asp Gln
661 GCG GCG TGC TGT ATC GTG GGT CAG AGT GAG CAG CTG GTT CCT GCG GAG GGA ATC CTA TAT
180 Ala Gly Cys Cys Ile Val Gly Gln Ser Glu Gln Leu Val Pro Ala Asp Gly Ile Leu Tyr
721 GCA GCC AGA GAT GTG ACA GCG ACC GTG GAG AGC CTG CCA CTC ATC ACA GCG TCC ATT CTC
200 Ala Ala Arg Asp Val Thr Val Asp Ser Leu Pro Leu Thr Thr Ala Ser Ile Leu
781 AGT AAG AAA CTC GTG GAG GGG CTG TCC GCT CTG GTG GTG GAG GTT AAG TTC GGA GGG GCC
220 Ser Lys Lys Leu Val Glu Gly Leu Ser Ala Leu Val Val Asp Val Lys Phe Gly Gly Ala
841 GCG GTC TTC CCC AAC CAG CAG GAG GCG GCG GAG CTG GCA AAG ACG GTG GGT GGT GCG GTG GGA
240 Ala Val Phe Pro Asn Gln Gln Gln Ala Arg Glu Leu Ala Lys Thr Leu Val Gly Val Gly
901 GCG AGC CTA GCG CTT CCG GTC GCG GCA GCG CTG ACC GCG ATG GAG AAG CCC CTG GGT CCG
260 Ala Ser Leu Gly Leu Arg Val Ala Ala Leu Thr Ala Met Asp Lys Pro Leu Gly Arg
961 TCG GTG GCG CAG GCG CTC GAG GTC GAG GAG GCG CTG CTC TGC ATG GAG GCG GCA GCG CCG
280 Cys Val Gly His Ala Leu Glu Val Glu Glu Ala Leu Cys Met Asp Gly Ala Gly Pro
1021 CCA GCA TTA AGG GAC CTG GTC ACC ACG CTC GGG GCG GCC CTG CTC TGG CTC ACG GCA CAC
300 Pro Asp Leu Arg Asp Leu Val Thr Thr Leu Gly Gly Ala Leu Leu Trp Leu Ser Gly His
1081 GCG GCG ACT CAG GCG CAG GCG GCT GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
320 Ala Gly Thr Gln Ala Gln Gly Ala Ala Arg Val Ala Ala Leu Asp Asp Gly Ser Ala
1141 CTT GCG CCG TTC GAG CCG ATG CCG GCG CAG GCG GTC GAT CCG GGT CTG GCG CCA GCG
340 Leu Gly Arg Phe Glu Arg Met Leu Ala Ala Gln Gly Val Asp Pro Gly Leu Ala Arg Ala
1201 CTG TCG TCG GGA AGT CCC GCA GAA GCG GCG CAG CTG CCG CCG GCG GAG CAG CAG
360 Leu Cys Ser Gly Ser Pro Ala Glu Arg Arg Gln Leu Leu Pro Arg Ala Arg Glu Gln Glu
1261 GAG CTG CTG GCG CCC GCA GAT GCG ACC GTG GAG CTG GTC CCG GCG CTG CCG CTG GCG CTG
380 Glu Leu Leu Ala Pro Ala Asp Gly Thr Val Glu Leu Val Arg Ala Leu Pro Leu Ala Leu

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1321 GTG CTG CAC GAG CTC GGG GCG GCG GCG AGC CCG GCT GGG GAG CCG CTC CCG CTG GGG GTG
400 Val Leu His Glu Leu Gly Ala Gly Arg Ser Arg Ala Gly Glu Pro Leu Arg Leu Gly Val
1381 GCG GCA GAG CTG CTG GTC GAG GTG GGT CAG AGG CTG CCG CCG GCG ACC CCG TGG CTC CCG
420 Gly Ala Glu Leu Leu Val Asp Val Gly Gln Arg Leu Arg Arg Gly Thr Pro Trp Leu Arg
1441 GTG CAC CCG GAG GCG CCC GCG CTC AGC GCG CCG CAG AGC GCG GCG CCG CAG GAG GCG CTC
440 Val His Arg Asp Gly Pro Ala Leu Ser Gly Pro Gln Ser Arg Ala Leu Gln Glu Ala Leu
1501 GTA CTC TCC GAG CCG GCG CCA TTC GCG CCC TCG CCC TTC GCA GAG CTC GTT CTG CCG
460 Val Leu Ser Asp Arg Ala Pro Phe Ala Ala Pro Leu Pro Phe Ala Glu Leu Val Leu Pro
1561 CCG CAG CAA TAA ACC TCC TTT GCG GCG AAA (A)n
480 Pro Gln Gln

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FIG. 1 Nucleotide sequence of PD-ECGF. The putative initiation codon is at nucleotides (nt) 124–126. An in-frame stop codon (nt 28–30) upstream of the initiation codon is marked with dots and a stop codon at nt 1,570–1,572 with an asterisk. The polyadenylation signal (nt 1,568–1,573) is underlined with an arrow. Regions corresponding to the prepared oligonucleotide probes 228, 231, 240, 258 and 259, are underlined.

**METHODS.** Total RNA was isolated from a human placenta of 22-gestational-weeks by the method of Han *et al.*<sup>21</sup>. A cDNA library was constructed in  $\lambda$ gt10 from the poly(A)<sup>+</sup> RNA following the method described by Watson *et al.*<sup>22</sup> with some modifications: following the first strand synthesis, oligo(dG) was added to its 3' end by terminal transferase; second strand synthesis was primed by addition of an external primer of oligo(dC), as well as internal RNA primers made by RNaseH. Five unique oligonucleotides were deduced from the amino-acid sequence by the method of Lathe<sup>23</sup> and prepared by an Applied Biosystems DNA synthesizer 381A. The oligonucleotide probes were labelled by end-tailing or primer extension. Independent clones (~300,000) from the human placenta  $\lambda$ gt10 cDNA library were screened with these probes separately following the method described by Ullrich *et al.*<sup>24</sup>. Three clones were found to be positive both with probes 228 and 240. The cDNA inserts were shown to be colinear by restriction mapping. The cDNA insert of  $\lambda$ PL8, with the longest insert of ~1.8 kb, was subcloned into M13mp18 and Bluescript (Stratagene). Deletion mutants were made by the sequential deletion method of Yanisch-Perron *et al.*<sup>25</sup>. Both strands of the cDNA were sequenced by the dideoxy-termination method<sup>26</sup>. Sequences that were difficult to determine by this procedure were examined by the Maxam-Gilbert method<sup>27</sup>.

FIG. 2 Amino-acid sequence of PD-ECGF (one-letter code). N-terminal sequence (—), tryptic fragments (---), staphylococcal protease V8 fragments (---) and a CNBr fragment (====) of PD-ECGF are indicated, as well as Cys residues (\*), a potential N-glycosoylation site (■), and a possible site of polymorphism (▲). Internal repeats are overlined.

**METHODS.** To prepare tryptic fragments, 40  $\mu$ g pure PD-ECGF<sup>2</sup> was desalted on a C4 narrow-bore reversed-phase column (Brownlee Aquapore BU-300; 2.1  $\times$  30 mm), eluted with 0.1% trifluoroacetic acid and a gradient of acetonitrile, and then dried in a Speedvac Concentrator and redissolved in 200  $\mu$ l 6 M guanidine-HCl, 0.25 M Tris-HCl, pH 8.5, 1 mM EDTA, containing 100  $\mu$ g dithiothreitol. The solution was flushed with N<sub>2</sub> for 20 s and left at room temperature for 3 h, at which time 2  $\mu$ l 4-vinyl-pyridine was added. After a further 3 h at room temperature, the sample was desalted by chromatography on the C4 column. The volatile solvent was removed as above and PD-ECGF was digested with TPCK-trypsin (Sigma; substrate/enzyme ratio, 50 (w/w)) in 0.1 M ammonium bicarbonate, containing 2 M urea, for 4 h at 37°C. The tryptic fragments were immediately loaded onto a C4 narrow-bore, reversed-phase HPLC column eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. Non-homogeneous peptides were re-run on HPLC narrow-bore reversed-phase column under different conditions. The column temperature was kept at 35°C, the flow rate was 100  $\mu$ l min<sup>-1</sup> and the effluents were monitored at 220 nm. Fractions were collected manually in polypropylene tubes. Peptides were also isolated from PD-ECGF fragmented with staphylococcal V8 protease or CNBr

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MAALMTPTGTGAPPAPGDFSGEGSQGLPDPSPPEPKQLPELIRMKRDGGRLSEADIRGFVAA 60
-----
VYNGSAAGAGI GAMLMAIRLRGMDLEETSVLTQALAQSGQOLEWPEAWRQOLVDKHSTGG 120
-----
VGDKVSLVLAPALAACGCKVPMISGRGLGHTGSLDKLESTPGFNVIQSPQMOVLDDOA 180
-----
GCCIVGQSEQLVPADGILYAARDVTATVDSLPLITASILSKKLVGLSALVVDVKFGGAA 240
-----
VFPNQEARELAKTLVGVSASLGLRVAAL TAMDKPLGRGVGHAEVEEALLCMQDAGPP 300
-----
DLRLVLTLLGALLWLSGHAGTQAQGAARVAALDDGSA LGRFERMLAAGQVDPLRALAR 360
-----
CSGSPAERRQLLPAREQEELLAPADGTVELVRALPLALVHLHAGRSRAGEPLRLGVG 420
-----
AELLVDVGQRLRRGTPWLRVHRDGPALSGPQSRALQEALVLSDRAPFAAPLFAELVLPQQ 482
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by similar methods. The amino-acid sequences of the peptides were determined by use of an automated gas-phase sequencer (Applied Biosystems Protein sequencer, model 470A, with an online PTH-analyser, model 120A).

hydrophobic signal sequence, suggesting that PD-ECGF is not a classical secretory protein. The sequence contains only one Tyr, which is probably not exposed on the surface of the molecule, as attempts to radiolabel PD-ECGF with  $^{125}\text{I}$  using the chloramine-T method resulted in a very low incorporation of radioactivity<sup>2</sup>. There is one potential *N*-glycosylation site, Asn 63. The PTH-amino-acid yield of the corresponding tryptic peptide decreased dramatically, just before this residue (Fig. 2). This could be due to the formation of a cyclic compound of the Asn residue and the following Gly residue<sup>7</sup>, which can occur only if the Asn residue is non-glycosylated. This, in combination with the  $M_r$  of PD-ECGF as determined by SDS-gel electrophoresis, compared with the  $M_r$  predicted from the cDNA clone, indicates that Asn 63 is not glycosylated. There are a total of seven Cys residues, indicating that the molecule contains at least one free thiol group.

### Southern and northern blotting

To obtain information about the structure of the human PD-ECGF gene, Southern blotting analysis of human DNA was performed. Hybridization with a PD-ECGF cDNA probe revealed single bands of 19 kb and 9.2 kb after digestion with *Hind*III and *Xba*I, respectively (Fig. 3a). This indicates that only one copy of the PD-ECGF gene is present in the human genome. Indeed, analysis of human genomic PD-ECGF clones supports this conclusion (K.H. *et al.*, manuscript in preparation). Human placenta poly(A)<sup>+</sup> RNA was examined by northern blotting; a single transcript of about 1.8 kb was recognized by the PD-ECGF cDNA probe (Fig. 3b). As the longest cDNA clone found,  $\lambda$ PL8, has an insert of about 1.8 kb, it probably represents a full-length copy of the PD-ECGF transcript.

### Expression of PD-ECGF cDNA

To verify the authenticity of the cDNA clone, it was expressed in NIH 3T3 cells. The  $\lambda$ PL8 insert was subcloned into the pLJ expression vector to give pLPL8J. This vector has Molony leukaemia virus long terminal repeat as a promoter to drive the cDNA transcription, as well as a neomycin-resistance gene as a selection marker<sup>8</sup>. pLPL8J and pLJ were introduced into the NIH 3T3 cells by the calcium phosphate co-precipitation method, and cell lines were selected by neomycin. Analysis of one cell line transfected with pLPL8J revealed growth-promoting activity for porcine aortic endothelial cells in the cell lysate, but not in the conditioned medium (Fig. 4a,b). The activity was completely neutralized by a specific rabbit antiserum against PD-ECGF, indicating that the activity was due to the synthesis of functionally active PD-ECGF by the cell line (Fig. 4a). The NIH 3T3 cell line transfected with only the pLJ vector did not contain any growth-promoting activity for porcine aortic endothelial cells. Furthermore, the cell lysate of the cell line transfected with pLPL8J was found to contain a 45K component in immunoblotting experiments in which the antiserum against PD-ECGF was used (Fig. 4c). The size of the product was similar to that of human PD-ECGF purified from platelets (Fig. 4c). These data show that the purified, sequenced and cloned PD-ECGF molecule is responsible for the observed biological effect on porcine endothelial cells. That PD-ECGF produced by the transfected NIH 3T3 cells remained inside the producer cells and was not secreted to any appreciable extent into the cell culture medium, is consistent with the lack of a signal sequence in the protein (Fig. 1). This is a property that PD-ECGF shares with acidic FGF (ref. 9) and basic FGF (ref. 10). It is not known whether there are alternative mechanisms to release these factors from their producer cells.

### Chemotactic activity of PD-ECGF

PD-ECGF was found to have a chemotactic effect on bovine aortic endothelial cells, with half-maximal activity at about  $1 \text{ ng ml}^{-1}$  (Fig. 5a). Consistent with its mitogenic target cell specificity for endothelial cells, PD-ECGF did not induce

smooth muscle cell migration. Controls using serum- and platelet-derived growth factor induced extensive smooth-muscle-cell migration in the same experiment. Checkerboard analysis revealed that endothelial cell migration induced by PD-ECGF was due to directed cell migration (chemotaxis) and not to random migration (chemokinesis) (Fig. 5b). The chemotactic activity of PD-ECGF was neutralized by PD-ECGF antibodies (Fig. 5c).

### Angiogenic activity of PD-ECGF

Two different approaches were taken to investigate whether PD-ECGF has angiogenic activity *in vivo*. First, we investigated the effect of PD-ECGF on the developing vascular system of the chick chorioallantoic membrane. Partially purified PD-ECGF consistently induced a strong angiogenic response (Fig. 6a), which was inhibited by antibodies against PD-ECGF (Fig. 6b). Thus, the angiogenic response is unlikely to be due to inflammation or other factors than PD-ECGF present in the preparation. Furthermore, pure PD-ECGF, at a dose of 50 ng, induced angiogenesis in the chorioallantoic membrane (data not shown).

Second, we investigated the effect of transfection of PD-ECGF cDNA into tumour cells on the vascularization of tumours formed by these cells in nude mice. NIH 3T3 cells transformed by an activated human *Ha-ras* gene<sup>11</sup> (termed a1-1 cells) were chosen as target cells and were transfected with a plasmid containing PD-ECGF DNA (pLPL8J), or with a control plasmid without insert (pLJ). After transfection, cells were selected by G418 resistance, and then injected subcutaneously into nude mice. After 2 weeks, tumours had grown to ~2 cm in diameter from both cell types; they were then collected and fixed in formaldehyde. A histological examination revealed that the tumours that developed from a1-1 cells, transfected with

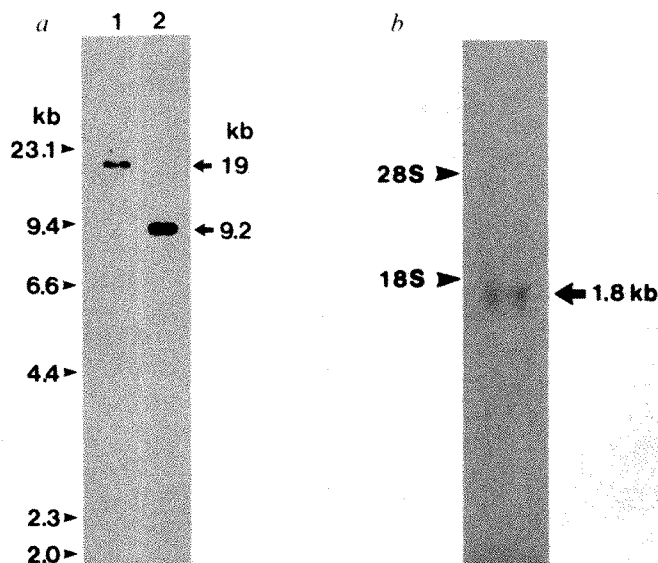


FIG. 3 a, Southern blot analysis of *Hind*III (lane 1) or *Xba*I (lane 2) digests of human DNA. b, Northern blot analysis of poly(A)<sup>+</sup> RNA from human placenta. Migration positions of ribosomal RNA markers are shown on the left. Blots were hybridized with PD-ECGF cDNA probes.

**METHODS.** High-molecular weight human genomic DNA was prepared from normal human leukocytes. Samples of  $10 \mu\text{g}$  each were digested by restriction enzymes, subjected to electrophoresis in agar and blotted on to nitrocellulose membranes (Schleicher and Schüll). Poly(A)<sup>+</sup> RNA ( $1 \mu\text{g}$ ), purified from human placenta of 22 weeks' gestation as described in the legend to Fig. 1, was electrophoresed in a 0.9% formalin-denaturing gel and blotted on to Hybond-N nylon filter (Amersham). Hybridization was performed in a solution of 50% formamide, 0.65 M NaCl, 0.1 M sodium PIPES, pH 6.8, 10% dextran sulphate,  $5\times$  Denhardt's solution, 0.1% SDS, 4 mM EDTA and  $100 \mu\text{g ml}^{-1}$  salmon sperm DNA at  $42^\circ\text{C}$  for 18 h, and then washed four times with  $2\times\text{SSC}$ , 0.2% sodium phosphate, 0.1% SDS for 20 min periods at  $50^\circ\text{C}$ .

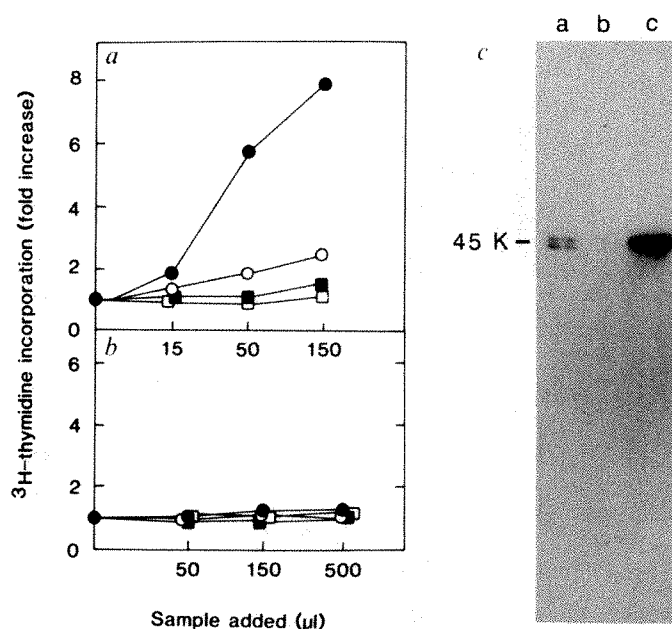
pLPL8J, contained a very high density of blood vessels (Fig. 6c), by contrast with tumours from a1-1 cells transfected with pLJ without cDNA insert, which contained only a few blood vessels (Fig. 6d). In conclusion, PD-ECGF has a potent angiogenic activity both in the chorioallantoic membrane assay and when expressed by tumour cells growing in nude mice.

## Discussion

Angiogenesis is an important process during, for example, embryogenesis, wound healing and organ regeneration. In addition, aberrant angiogenesis occurs in several pathological conditions, such as in tumour growth, in certain retinopathies, and in rheumatoid arthritis. Angiogenesis is a complex process that involves several steps, including migration and proliferation of endothelial cells<sup>12</sup>. The best characterized of the factors that

have been found to stimulate angiogenesis are FGFs, a family of heparin-binding endothelial cell mitogens that were originally purified from neural tissues<sup>3,4</sup>. Additionally, transforming growth factor (TGF)- $\alpha$ <sup>13</sup>, TGF- $\beta$ <sup>14,15</sup>, tumour necrosis factor- $\alpha$ <sup>16,17</sup> and angiogenin<sup>18</sup> have been found to have angiogenic activity. PD-ECGF, acidic and basic FGFs, and TGF- $\alpha$  are the only factors that have been shown to stimulate endothelial cells both *in vitro* and *in vivo*<sup>3,4,13</sup>. The target cell specificities of these factors, however, differ: FGFs and TGF- $\alpha$  stimulate proliferation of a wide variety of cells including fibroblasts, whereas PD-ECGF so far has not been found to stimulate any cell type other than endothelial cells. Furthermore, the deduced primary structure of PD-ECGF shows no similarity with other known proteins. Thus, the biological and structural properties of PD-ECGF indicate that it is a novel type of angiogenic factor that

FIG. 4 Biosynthesis of PD-ECGF in NIH 3T3 cells transfected with pLPL8J. Growth factor activity from a cell lysate (a) and the conditioned medium (b) of NIH 3T3 cells, transfected with PD-ECGF cDNA (NIH3T3-pLPL8J; circles) or with the corresponding vector without insert (NIH3T3-pLJ; squares) in the absence (filled symbols) and presence (open symbols) of PD-ECGF antibodies<sup>2</sup>. PD-ECGF protein was also visualized by immunoblotting using PD-ECGF antiserum (c); lane a, PD-ECGF purified from human platelets; lane b, cell lysate for NIH 3T3-pLJ cells; lane c, cell lysate from NIH3T3-pLPL8J cells. **METHODS.** NIH 3T3 cells transfected with pLJ or with pLPL8J were grown to confluence in 10-cm cell-culture dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and antibiotics. The cells were then cultured for 24 h in serum-free conditions in DMEM supplemented with 1% bovine serum albumin, 7.8  $\mu\text{g ml}^{-1}$  cholesterol, 5.5  $\mu\text{g ml}^{-1}$  oleic acid, 8  $\mu\text{g ml}^{-1}$  L- $\alpha$ -phosphatidylcholine and 0.2  $\text{mg ml}^{-1}$  transferrin. After collecting the conditioned media, cells were washed twice in PBS and then scraped into 1 ml PBS. Following careful resuspension, a cell lysate was prepared by disrupting the cells by three cycles of freezing and thawing, followed by centrifugation at 25,000g for 30 min and collection of the supernatants. Growth-promoting activity was measured by the incorporation of <sup>3</sup>H-thymidine into porcine aortic endothelial cells<sup>1</sup> in the absence or presence of 2  $\mu\text{g ml}^{-1}$  PD-ECGF antibody purified by protein A-Sepharose<sup>2</sup>. For immunoblotting, cell lysates were prepared by washing and resuspending the cells in PBS containing 1 mM phenylmethylsulphonyl fluoride (Sigma), 150 KIU aprotinin (Sigma) and 10 mM EDTA. The lysates and 100 ng pure PD-ECGF<sup>2</sup> were subjected to SDS-gel electrophoresis in 10–18% gradient polyacrylamide gel under reducing conditions<sup>28</sup>. After transfer to a nitrocellulose membrane, immunoblotting was performed as described<sup>29</sup>, using a 1:50 dilution of PD-ECGF antiserum<sup>2</sup>. <sup>125</sup>I-labelled staphylococcal protein A, bound to antibodies on the blot, was visualized by autoradiography. PD-ECGF



purified from platelets occurs as a doublet of about 45K, probably as a result of proteolysis during preparation<sup>2</sup>.

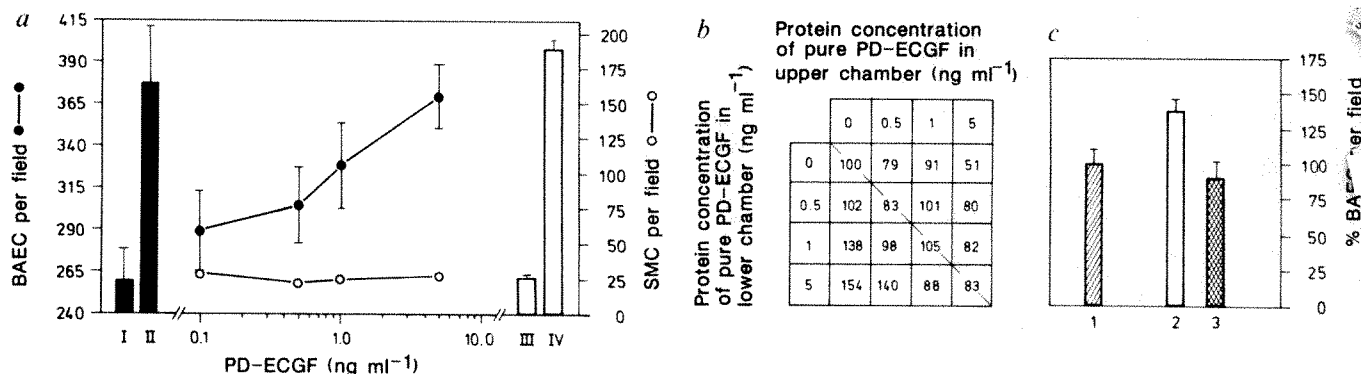


FIG. 5 Chemotactic activity of PD-ECGF. a, The migration of bovine aortic endothelial cells (BAEC; closed circles and left ordinate) and smooth muscle cells (SMC; open circles and right ordinate) towards different concentrations of PD-ECGF. Controls are shown as a histogram: I, BAEC medium only; II, BAEC medium plus 10 ng ml<sup>-1</sup> basic FGF; III, SMC medium only; IV, SMC medium plus 5% FCS. b, Checkerboard analysis of BAEC migration. The data represent cell number per field (%). Standard deviations were below 15%. The values below the diagonal indicate chemotaxis. c, The chemotactic activity of PD-ECGF is neutralized by PD-ECGF antibodies: 1, buffer control; 2, pure PD-ECGF (5 ng ml<sup>-1</sup>); 3, pure PD-ECGF (5 ng ml<sup>-1</sup>) and anti-PD-ECGF

antibodies (300 ng ml<sup>-1</sup>) (purified by protein A-Sepharose).

**METHODS.** Chemotaxis assays of BAEC and SMC were performed in 48-well microchambers as described<sup>30</sup>. Gelatin and fibronectin coating of the Nucleopore filters was necessary for BAEC adhesion. Cells (18,000 BAEC or 25,000 SMC per well) were added to the upper wells. Serum-free DMEM was used for SMC migration, and DMEM supplemented with 1% FCS for BAEC migration. The number of cells that had migrated during a 5 h incubation period to the lower surface of the filter were counted in three high-power fields. All experiments were done in triplicate.



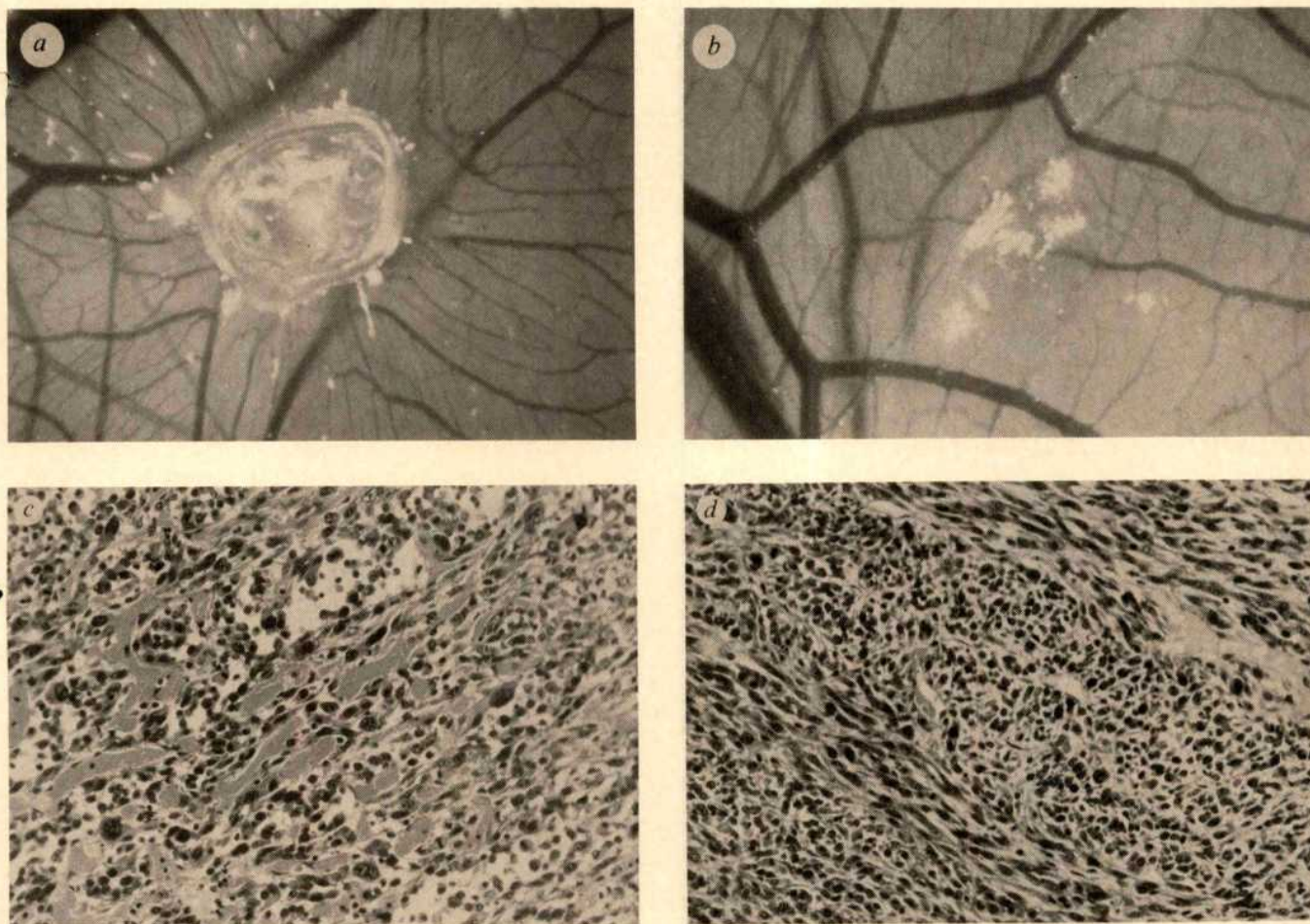


FIG. 6 Angiogenic properties of PD-ECGF. *a*, and *b*, The induction of angiogenesis in the chick chorioallantoic membrane. *a*, Partially purified PD-ECGF (purified through the hydroxylapatite step<sup>2</sup>, 1% pure, 1.2 mg of protein ml<sup>-1</sup>; 5  $\mu$ l per methylcellulose disk) induced angiogenesis on the chick chorioallantoic membrane (89% positive results,  $n$ (number of embryos)=65). *b*, Partially purified PD-ECGF incubated for 20 min with anti-PD-ECGF antibodies (2.5  $\mu$ l each solution) (59% negative results,  $n$ =22). In addition, 50 ng pure PD-ECGF induced angiogenesis (93% positive results,  $n$ =15). Test substances were incorporated into methylcellulose disks (10  $\mu$ l) and transplanted onto 9-day-old chorioallantoic membranes as described<sup>31,32</sup>. The assays were analysed daily for 2 days. Disks were visible by their light reflections. *c* and *d*, The angiogenic effect of PD-ECGF after

expression by tumour cells growing in nude mice. a1-1 cells were transfected with pLJ (control) or pLPL8J (carrying a PD-ECGF insert) by electroporation (Biorad, Gene Pulser). Transfected cell clones were selected by G418 resistance. All a1-1 clones transfected with pLPL8J were found to have growth-promoting activity on porcine aortic endothelial cells, which could be inhibited by PD-ECGF antibodies (data not shown); a1-1 cells transfected with pLJ had no such activity. a1-1 cells transfected with pLPL8J (*c*) and cells transfected with pLJ (*d*) were injected subcutaneously at a cell number of  $1 \times 10^5$  into nude mice and tumours of ~2-cm diameter developed in ~2 weeks. Tumours were collected, fixed with formaldehyde, stained with hematoxylin-eosin and examined with light-microscope. The photographs represent an 825-fold magnification.

may act specifically on endothelial cells. The finding that PD-ECGF is present in the placenta is intriguing, since angiogenesis is an important process in the development of this organ.

The restricted target cell specificity of PD-ECGF and the fact that platelets are a main source of it, suggest that PD-ECGF has a role in maintaining the integrity of the blood vessels. After injury to the endothelial cell layer of vessels, platelets aggregate at the subendothelial space. Platelet-release products, such as platelet-derived growth factor, are thought to be involved in the development of atherosclerotic lesions by stimulating migration and proliferation of smooth muscle cells and fibroblasts in the arterial media<sup>19,20</sup>. Although the precise mechanism of secretion of PD-ECGF is not known at present, it is possible that PD-ECGF counteracts the atherogenic effect of other platelet products by promoting repair of the endothelial cell layer. The availability of functionally active recombinant PD-ECGF (Fig. 4), combined with a high yield purification method<sup>2</sup>, cDNA clones (Fig. 1) and specific antibodies<sup>2</sup>, now makes it possible to address questions related to the function of PD-ECGF *in vivo*. □

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## LETTERS TO NATURE

## A marked concentration of galaxy clusters: is this the origin of large-scale motions?

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THE distances and velocities of 400 elliptical galaxies<sup>1</sup>, out to redshifts equivalent to recession velocities of  $\sim 6,000 \text{ km s}^{-1}$ , suggest that the peculiar velocities obtained by subtraction of the general cosmological expansion are best fitted by a flow induced by a 'great attractor'<sup>2</sup>, a large mass of  $5.4 \times 10^{16} M_{\odot}$  and centred on galactic longitude  $l = 307^{\circ}$  and latitude  $b = 9^{\circ}$  at a distance  $R_m = 4,350 \pm 350 \text{ km s}^{-1}$ . A redshift survey of  $\sim 900$  galaxies<sup>3</sup> shows that the excess galaxy number counts in this direction are due to two substantial concentrations of galaxies at recession velocities  $v \approx 3,000 \text{ km s}^{-1}$  and  $4,500 \text{ km s}^{-1}$ . Here we show that in roughly the same direction there is also a very rich concentration of galaxy clusters which may have a considerable dynamical influence. The estimated redshift distances of these clusters range from  $3,000$  to  $20,000 \text{ km s}^{-1}$ , with a main complex at  $v \approx 14,000 \text{ km s}^{-1}$ . The barycentre of this concentration lies  $\sim 25^{\circ}$  away from the cosmic microwave background dipole<sup>4</sup>, and  $\sim 10^{\circ}$  away from the latest reported position of the Great Attractor<sup>5,6</sup>.

The distribution of clusters of galaxies is of fundamental significance in understanding the matter distribution, in explaining the origin of the observed peculiar velocity field<sup>7,2</sup>, and in estimating both the local peculiar acceleration vector and the distance at which the Hubble flow becomes unperturbed<sup>8</sup>. Although their number is relatively small, the clusters, being peaks of the density field, might avoid the problems that some other all-sky density tracers (for example, IRAS galaxies<sup>9–12</sup>) have in describing the large-scale density field.

We have analysed the southern part of the new Catalogue of Rich Clusters of Galaxies<sup>13</sup> (hereafter ACO), which lists 1,638 clusters with declination  $\delta \leq -17^{\circ}$ . This catalogue lists redshifts for 145 clusters. The incompleteness of the sample with respect to redshift means that, contrary to what has been done in the Northern Hemisphere<sup>14</sup>, it is not sufficient to use those few clusters with known velocity to map the large-scale distribution of clusters in the Southern Hemisphere. We have therefore used 143 redshifts ( $z$ ) to estimate a linear relation between  $\log [D_L(z)] + 0.2K(z)$  and the magnitude of the tenth brightest member, including also a correction for the cluster richness (Scott effect). Here,  $D_L(z)$  is the luminosity distance, assuming cosmological deceleration  $q_0 = 1/2$ , and  $K(z)$  is the K-correction for E and S0 galaxies<sup>15</sup>. We find that the relation has a dispersion

$\sigma_{\text{sample}} \approx 0.11$  in  $\log [D_L(z)] + 0.2K(z)$ , corresponding to  $\Delta z/z \approx 0.25$  for small  $z$ . In deriving this relation, two clusters have been excluded because their redshift appeared to be inconsistent (beyond three standard deviations) with the magnitude given in the catalogue. Hereafter co-moving distances  $d = D_L(z)/(1+z)$  will always be scaled by the value of  $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$  and quoted in  $\text{km s}^{-1}$ .

A preliminary identification of candidate superclusters was made using a percolation algorithm on the reference catalogue, that is, the catalogue with distances  $d = \langle d \rangle$ , where the average is derived from a gaussian distribution in  $\log [D_L(z)] + 0.2K(z)$  with variance  $\sigma_{\text{sample}}^2$ . The statistical robustness of these structures was verified on 100 random catalogues in which the estimated redshift for each cluster was drawn according to the previous distribution.

A detailed description of the  $z$  estimates and of the supercluster candidates from the ACO catalogue, as well as of their robustness and membership, will be given elsewhere (R.S. *et al.*,

TABLE 1 Clusters within the region delimited by  $12^{\text{h}}40^{\text{m}} \leq \alpha \leq 14^{\text{h}}08^{\text{m}}$ ,  $-41^{\circ} \leq \delta \leq -25^{\circ}$ , with a mean co-moving distance  $\langle d \rangle \leq 20,000 \text{ km s}^{-1}$ .

Identification no.	$\alpha$	$\delta$	$\langle d \rangle$	$N_{\text{gal}}$
<b>1736</b>	13 h 24.3 min	$-26^{\circ}54'$	10,225	104
<b>3526</b>	12 h 46.1 min	$-41^{\circ}02'$	3,271	33
<b>3528</b>	12 h 51.6 min	$-28^{\circ}45'$	15,922	70
3530	12 h 52.9 min	$-30^{\circ}05'$	13,494	34
3532	12 h 54.6 min	$-30^{\circ}06'$	14,892	36
3535	12 h 55.1 min	$-28^{\circ}13'$	16,902	30
<b>3537</b>	12 h 58.3 min	$-32^{\circ}10'$	4,945	35
3542	13 h 05.9 min	$-34^{\circ}18'$	17,108	45
3553*	13 h 16.4 min	$-36^{\circ}55'$	15,141	36
3554	13 h 16.7 min	$-33^{\circ}13'$	17,220	59
3555	13 h 18.0 min	$-28^{\circ}43'$	17,645	61
3556	13 h 21.3 min	$-31^{\circ}24'$	16,916	49
3557	13 h 22.1 min	$-28^{\circ}37'$	16,628	36
<b>3558*</b>	13 h 25.1 min	$-31^{\circ}14'$	13,948	226
<b>3559*</b>	13 h 27.1 min	$-29^{\circ}16'$	13,641	141
3560*	13 h 19.0 min	$-32^{\circ}58'$	12,349	184
<b>3562*</b>	13 h 30.7 min	$-31^{\circ}25'$	14,422	129
3564*	13 h 31.5 min	$-34^{\circ}58'$	12,406	53
<b>3565</b>	13 h 33.8 min	$-33^{\circ}43'$	3,241	64
3566*	13 h 36.1 min	$-35^{\circ}18'$	15,361	100
3570	13 h 43.9 min	$-37^{\circ}40'$	10,851	31
3571*	13 h 44.6 min	$-32^{\circ}37'$	15,259	126
3572*	13 h 45.3 min	$-33^{\circ}08'$	12,992	49
<b>3574</b>	13 h 46.3 min	$-30^{\circ}03'$	4,183	31
3575*	13 h 49.7 min	$-32^{\circ}38'$	13,597	49
3577*	13 h 51.5 min	$-27^{\circ}36'$	15,152	103
3578	13 h 54.7 min	$-24^{\circ}29'$	9,969	52
3581	14 h 04.6 min	$-26^{\circ}47'$	11,931	42

Clusters with identification no. in boldface have measured redshifts listed in the ACO catalogue. After this work was completed, we found measured redshifts<sup>26</sup> for four of the clusters with distance estimates. The measured values and the estimated ones are in agreement within the errors.

\* Clusters tentatively belong to the main complex cited in the text (value of the percolation radius  $R = 1,500 \text{ km s}^{-1}$ ).

manuscript in preparation). Of course, because of the uncertainty in our distance estimates, some numerical details in the following discussion may change after observed redshifts become available.

A remarkable concentration<sup>16</sup> of clusters in the direction of Centaurus is clearly evident in Fig. 1, which shows an all-sky projection of all the ACO and Abell clusters with a measured or estimated distance  $< 20,000 \text{ km s}^{-1}$ . We have derived an estimate of the overdensity of clusters in this region, which we call the  $\alpha$ -region, in the following way. The ACO catalogue covers 1.38 steradian in the latitude range  $20^\circ \leq |b| \leq 40^\circ$ . In this area it contains 96 clusters up to a distance of  $25,000 \text{ km s}^{-1}$ . Subtracting the number of clusters and the corresponding volume of the  $\alpha$ -region (28 clusters in 0.11 steradian up to a distance of  $20,000 \text{ km s}^{-1}$ ), we retain 68 clusters in a volume of  $\sim 7 \times 10^6 h^{-3} \text{ Mpc}^3$ , corresponding to a density of  $\sim 1 \times 10^{-5} h^{-3} \text{ clusters Mpc}^{-3}$ . This would imply an expected number of 2.9 clusters in the  $\alpha$ -region, corresponding to an observed overdensity of about a factor of 10 with respect to the mean volume density of all the other ACO clusters at a comparable galactic latitude (see Fig. 2). A possible source of error in the determination of the mean volume density of clusters (in addition to the statistical error which amounts to  $\sim 12\%$ ) could, in principle, derive from an incompleteness of the catalogue near the edge of the volume that we have used. We are, however, confident that this introduces only a minor uncertainty in our estimate, for two reasons. First, it is claimed<sup>13</sup> that the ACO catalogue is "nominally complete to  $z = 0.2$  for clusters with populations of 30 or more galaxies in the magnitude range  $m_3$  to  $m_{3+2}$ "; second, an analysis of the density of the ACO clusters as a function of distance (R.S. *et al.*, manuscript in preparation) shows that, after excluding the clusters in the  $\alpha$ -region, there is no evidence of a significant decrease of the density of clusters with richness  $R \geq 0$  up to at least  $R = 30,000 \text{ km s}^{-1}$ . In fact, similar values for the mean density of clusters are derived by using the volumes within  $R = 30,000 \text{ km s}^{-1}$  (130 clusters) or  $R = 35,000 \text{ km s}^{-1}$  (190 clusters).

As seen in Fig. 2, the distribution with depth of the clusters in the  $\alpha$ -region is not uniform, but is peaked at  $\langle d \rangle \approx 4,000 \text{ km s}^{-1}$  and at  $\langle d \rangle \approx 14,000 \text{ km s}^{-1}$ . The complex formed by the clusters denoted by '\*' among those listed in Table 1, appears to be worthy of future, detailed study. This candidate supercluster consists of a number of members similar to that of the Corona Borealis supercluster<sup>17</sup>, but has the advantage of being almost two times closer and two times more dense in clusters with richness  $R \geq 1$  than the Corona Borealis supercluster.

We are wary of using the spherical infall model to estimate the possible dynamical influence of the listed clusters, and have computed the peculiar acceleration,  $A_p$ , induced on the Local Group (LG) by the clusters listed in Table 1 according to  $A_p = A - \langle A \rangle$ . Here  $\langle A \rangle$  is the acceleration expected from the mean density of clusters in the same volume. It amounts to  $< 10\%$  of  $A$ , the acceleration induced on the LG by the observed clusters, which has been computed as<sup>18</sup>  $A = G \sum_i M_i \hat{r}_i / r_i^2$  where the mass of each cluster ( $M_i \equiv M_{R=2}(N_i^{\text{gal}}/106)$ ) is weighted by the number of galaxies in the cluster,  $N_i^{\text{gal}}$ , and is expressed in terms of the typical values of an  $R=2$  Abell cluster (that is, Coma, for which  $N_i^{\text{gal}} = 106$ ). We also have to assume pure Hubble flow for the proper distance,  $r_i = d_i H_0 / (1+z)$ , and obviously this can introduce systematic errors because of the presence of coherent peculiar flows.

About half the total computed acceleration is due to the nearest chain of four clusters ( $\langle d \rangle \approx 4,000 \text{ km s}^{-1}$ , consistent with the quoted distance of the Great Attractor) and the rest is due to the combined pull of the more distant complexes (see Fig. 2), which are dominated by the one at  $\langle d \rangle \approx 14,000 \text{ km s}^{-1}$  (eleven clusters denoted by '\*' in Table 1). This could help explain part of the 'large' peculiar velocity of the Centaurus cluster<sup>6</sup> and is consistent with the present lack of detection<sup>2</sup> of a reversed sign for the peculiar flow beyond  $4,500 \text{ km s}^{-1}$ . From 1,000 simulations, in which the estimated redshifts of the clusters were randomized around their nominal value, the standard error on the positively skewed distribution of the modulus of  $A$  is  $\leq 5\%$  whereas, given our narrow cone region, the direction

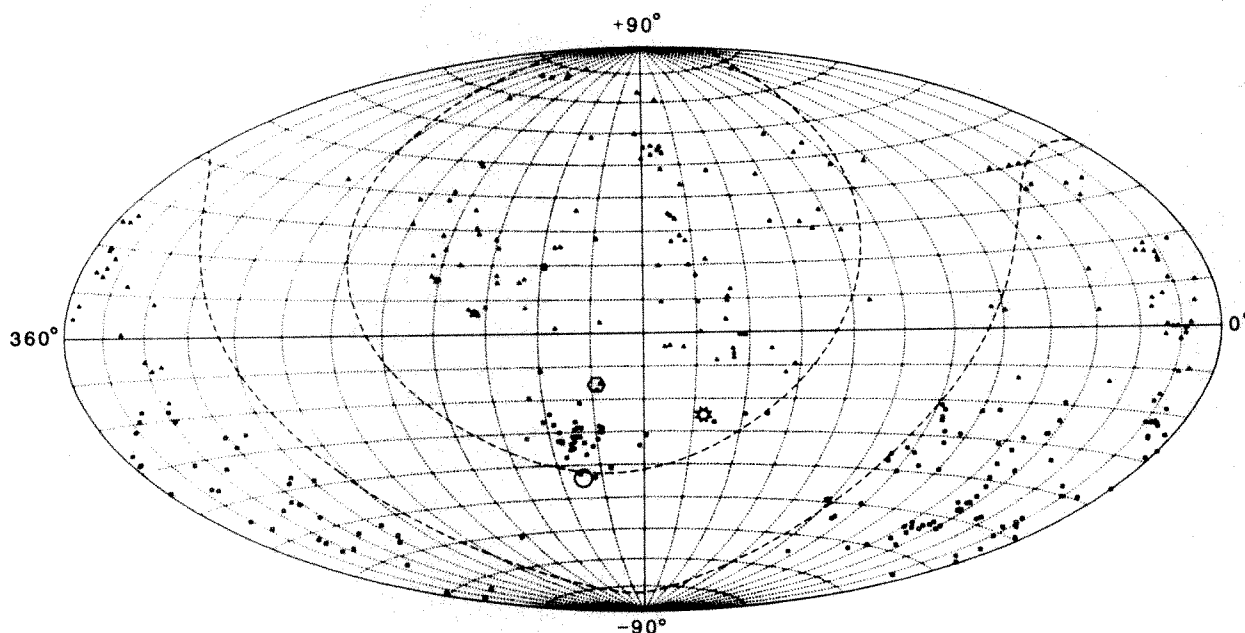


FIG. 1 Equi-area projection in right ascension ( $\alpha$ ) and declination ( $\delta$ ) of all the rich clusters with measured or estimated distances within  $20,000 \text{ km s}^{-1}$ . Triangles and squares represent Abell and ACO clusters respectively (some clusters are listed in both catalogues). The star shows the direction of the cosmic microwave dipole<sup>4</sup>, the empty circle shows the

latest direction of the Great Attractor<sup>5,6</sup>, and the empty hexagon shows the direction of the peculiar flow<sup>7</sup>. Between the latter two symbols the cluster concentration discussed in the text is clearly visible. Dashed lines indicate the avoidance zone due to the galactic plane ( $|b| \leq 20^\circ$ ).



almost coincides with the clusters' barycentre, being always within one degree from right ascension  $\alpha = 13^{\text{h}}26^{\text{m}}$  and  $\delta \approx -33^\circ$ . This agrees very well with one ( $\alpha = 13^{\text{h}}20^{\text{m}}$ ,  $\delta = -33^\circ$ ) of the possible directions for the Supergalactic Centre<sup>2</sup>. It is also close to a previously reported quadrupolar distortion of the peculiar flow<sup>19</sup>, while most of the optical dipole signal is due to a patch of sky also centred on the same zone<sup>20</sup>. Indeed, although the close agreement of the latter dipole with that of the cosmic microwave background favours a 'local' (within  $\sim 4,000 \text{ km s}^{-1}$ ) convergence of the peculiar acceleration, its contribution to the infall towards Centaurus<sup>20</sup>,  $v_p^{\text{dip}} \sim 250 \text{ km s}^{-1}$ , seems to account for only half of the observed one, again in good agreement with our above estimate.

If we now make the extreme hypothesis that the component of our peculiar velocity,  $v_p \approx 570 \text{ km s}^{-1}$ , towards this direction is entirely due to the  $A_p$  derived above, by using the relation<sup>18</sup>  $v_p = \frac{2}{3} H_0^{-1} \Omega_0^{-0.4} A_p$ , we have

$$M_{(R=2)} \approx 5 M_{15} h^{-1} \left( \frac{\Omega_0}{0.2} \right)^{0.4} \left( \frac{v_p}{570 \text{ km s}^{-1}} \right) \quad (1)$$

where  $M_{15} \equiv 10^{15} M_\odot$ .

This shows that in an open universe it would be possible to ascribe the whole required peculiar velocity to the observed clusters if the mass overdensity of an  $R=2$  cluster were  $\sim 5 M_{15}$ . Recent estimates<sup>21</sup> give the mass of the Coma cluster as  $\leq 3 M_{15}$ . The mass required is undoubtedly high, but the nominal value from equation (1) can be significantly lowered if the overdensity of clusters reported here extends into the avoidance region (see Fig. 1), and if, because of the incompleteness at such low galactic latitudes, many more clusters are present in the region we consider; according to a probability law  $P(|b|) = 10^{[-0.3(\csc |b| - 1)]}$ , one is likely to have missed half of the clusters with  $20^\circ \leq |b| \leq 40^\circ$ . Moreover, the required mass is not simply the mass confined within the Abell radius, but may be associated with larger galaxy aggregates in which the clusters are embedded, as suggested by the galaxy-cluster cross-correlation<sup>22,23</sup>. Similarly, if we divide the mass estimates<sup>17</sup> for the Corona Borealis Supercluster,  $M_{\text{SC}} \approx 26 M_{15}$ , by the number of members ( $N_{\text{cl}} = 6$  and weighted as above by the number of galaxies<sup>24</sup>) we obtain  $M_{(R=2)} \approx 6.6 M_{15}$ , which is in good agreement with the value derived from equation (1). Of course, this is not the end of the game because a biased distribution of matter would not be consistent with our assumption of clusters as tracers of the mass distribution, although recent estimates<sup>25</sup> seem to indicate a global biasing factor not very far from unity. A value of  $\Omega_0 = 1$  could still be made consistent with the constraints found from equation (1) either by boosting the estimate for  $M_{(R=2)}$  or, more realistically, by not requiring that the peculiar velocity be entirely due to a 'forward' gravitational pull. Underdensities and other great, comparable aggregates (Perseus-Pisces, or another new candidate supercluster similar to the one we present here but at larger distance (R.S. *et al.*, manuscript in preparation)) may have significant dynamical influences.

No definite conclusions can be drawn without a more complete picture of our surroundings. It would be of great interest to determine whether clusters of galaxies are partly responsible for the overall dynamics or, more unlikely, if we are led to infer the existence of very large mass condensations<sup>2</sup> ( $M_{\text{GA}} \approx 54 M_{15}$ ) in which no very rich clusters are seen<sup>3</sup>. This, if not attributed to highly specific initial conditions, might imply the existence of a physical process which would have coherently prevented cluster—but not galaxy—formation on scales of  $50\text{--}100 h^{-1} \text{ Mpc}$ .

Detailed study of this region is very important, not only because no similar nearby concentration of clusters exists elsewhere, but also because of its direction. It is hard to interpret as a mere coincidence the fact that such a concentration of clusters of galaxies is so close in the sky to both the direction of the cosmic microwave background dipole and that derived

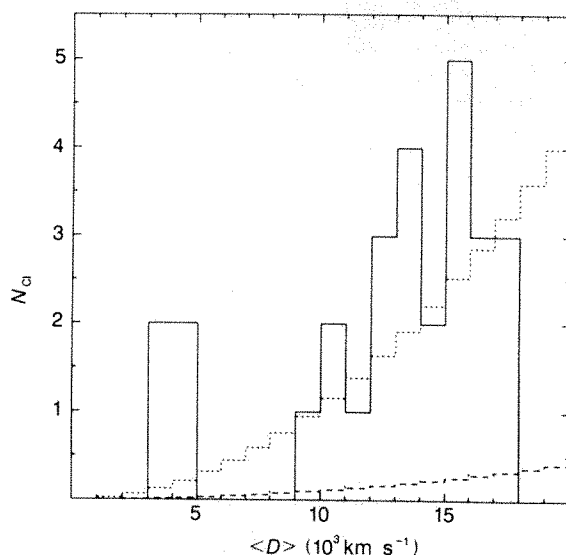


FIG. 2 Distribution in distance of the 28 clusters from Table 1 (solid line). The short-dashed line shows their expected distribution if they were uniformly distributed. The long-dashed line shows the distribution expected from the average cluster density.

from the peculiar motions of galaxies with respect to the Hubble flow. But it must be said that the large-scale mass fluctuation necessary if the whole peculiar velocity is due to the clusters in Table 1 would be difficult to accommodate in many current cosmological models.

Further studies of clusters in this area with intrinsic distance estimates should eventually find the distance at which the peculiar flow reverses its sign, thus delineating the region which contains the bottom of a great potential well (assuming that this is the major cause of the reported peculiar velocities (A. A. Starobinsky, preprint)). The concentration discussed here appears to be the best candidate for the bottom of such a well because of both the number of clusters present and their particular richness. □

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# Asymmetry of the envelope of supernova 1987A

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THE supernova SN1987A in the Large Magellanic Cloud has been observed by high-angular-resolution speckle interferometry since 25 March (30 days after the explosion) with the 4-m telescope at the Cerro Tololo Interamerican Observatory. These observations have provided a number of results that may be central to a detailed understanding of this unique event. Data obtained on 25 March and 2 April 1987 revealed a second bright 'companion' source separated from the supernova by 60 milliarcseconds and less than three magnitudes fainter than the supernova<sup>1</sup>. Measurements of the average diameter of the supernova envelope have been made from data recorded from March 1987 to April 1988<sup>2</sup>. Here we present a more detailed analysis of these data, which shows that the expanding envelope is asymmetric. This asymmetry has been observable since June 1987. The ratio between the minor and major axes of the envelope profile is about 2–3, and the position angle of the major axis is  $20^\circ \pm 5^\circ$ , consistent with results reported from polarization measurements. The major axis is aligned with the position angle of the companion to the supernova.

Speckle observations at optical wavelengths have been carried out using the PAPA detector<sup>3</sup>, a two-dimensional photon-counting sensor which records a catalogue of successive photon positions. The detector currently has a maximum data recording rate of 100,000 photons  $s^{-1}$  in a field of  $512 \times 512$  pixels. The speckle camera uses a fore-optics package which provides magnification of the image, so that the diffraction-limited scale of the telescope is matched to the pixel size of the camera, narrow-band optical filtering, which yields temporal coherence sufficient to allow interference over the path-length errors introduced by atmospheric aberrations, and atmospheric-dispersion correction, using a computer-controlled prism compensator. The digital output of the camera, in the form of photon addresses, is encoded on a video carrier and recorded with a VCR. The

stored addresses are recovered in the laboratory and unpacked for the speckle processing.

The photon addresses are grouped into frames, with a short time per frame relative to the atmospheric correlation time. This approach allows framing of the data for maximization of the signal-to-noise ratio in the integrated result; typical frame times range from 2 to 20 ms. Individual frames are built from the photon list by incrementing the number in the array position corresponding to the address of each detected photon. Corrections for the camera sensitivity (flat fielding) are made on the resulting frames. The Fourier transform for each frame is then calculated and accumulated into the power spectra and the complex correlation arrays which are required for diameter measurement and speckle image reconstruction using our version of the Knox-Thompson image-reconstruction algorithms<sup>4</sup>. Compensation for the atmospheric and telescope transfer function is accomplished by observing an unresolved comparison star immediately before and after the object observations. This star is chosen to be as close in angular position to the object as possible so that its atmospheric statistics are similar. Deconvolution by the reference star enhances the amplitudes of the high angular frequencies in the reconstruction. A detailed treatment of the algorithms and data processing is discussed elsewhere<sup>4</sup>.

Data sets on SN1987A and other stars result in diameter determinations with a precision of better than one milliarcsec. These measurements are made by fitting the integrated power spectra to the power spectrum of a uniform disk<sup>2</sup>, and, depending on the signal-to-noise ratio, can give results with resolution exceeding the diffraction limit of the telescope. Further analysis of the speckle data recorded between June 1987 (95 days after the explosion) and April 1988 (411 days after the explosion) shows that the expanding shell is elongated<sup>5</sup>. This asymmetry is detected from data recorded at several wavelengths between 442.0 nm and 850.0 nm. Figures 1a, c, e and g show the power spectra of SN1987A obtained from the data recorded near the centre of the H $\alpha$  emission line in May–June 1987, November 1987, February–March 1988 and April 1988. These power spectra are elongated, with a major axis corresponding to a position angle (PA) in image space of  $20^\circ$  (or  $200^\circ$ )  $\pm 5^\circ$ . Similar departures from circular symmetry are seen in the power spectra obtained from data recorded in several different wavelengths. By fitting the power spectra of uniform-brightness ellipses to the observed power spectra, we determined the lengths of the

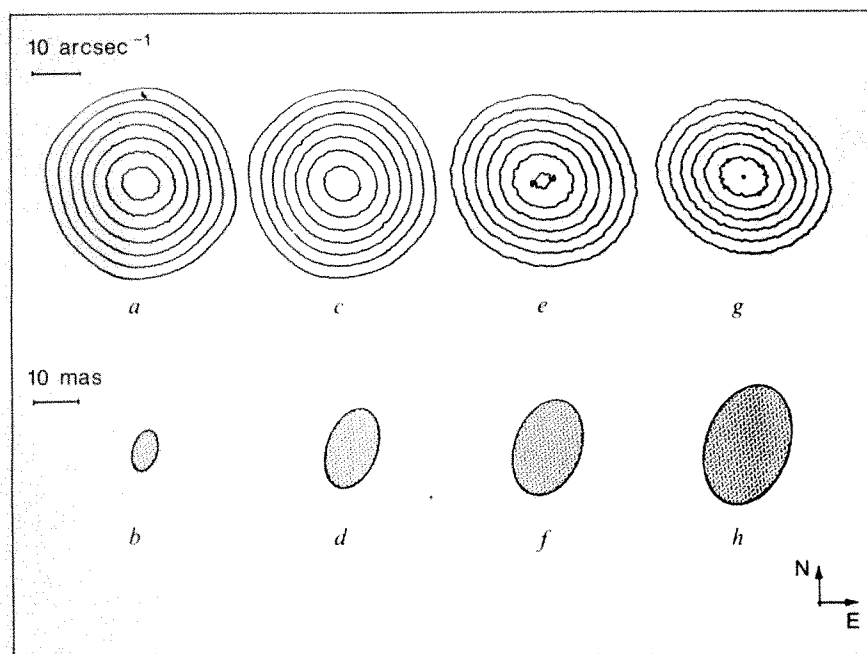


FIG. 1 Speckle power spectra of SN1987A recorded in H $\alpha$  at four different epochs. (a, c, e, g) and ellipses showing schematically the size and orientation of the envelope at each epoch (b, d, f, h). a, b, June 1987; c, d, November 1987; e, f, February 1988; g, h, April 1988.

major and minor axes, and their position angles. All of the measurements gave the same PA within the error bars. A summary of the measurements is given in Table 1. In contrast, data recorded on comparison stars give symmetric power spectra and symmetric images with measured angular diameters of less than 3 mas.

The ratio of the minor to major axis of the supernova's envelope is about 2–3 for all four data sets. The apparently increasing asymmetry in the supernova's power spectra shown in Fig. 1 is due to the increasing angular size of the supernova relative to the telescope's diffraction limit. Figures 1*b*, *d*, *f* and *h* show the ellipses whose Fourier transforms are fitted to the power spectra at the various epochs.

True images of SN1987A and its comparison star,  $\nu$  Vol, were reconstructed using Knox–Thompson speckle-imaging techniques<sup>4</sup>. Figure 2 shows the images from data recorded in April 1988 at 533.0 nm (10-nm bandpass) and H $\alpha$  (10-nm bandpass). The elongation of the supernova images, along an axis inclined at a position angle of 20° (or 200°), is quite evident despite the fact that its angular size in April was only slightly greater than the telescope's diffraction limit. Because the size of the image was so close to the telescope diffraction limit, the size of the axes were determined from power spectra, not from reconstructed images. The images also appear to be somewhat brighter in the south-west direction. The  $\nu$  Vol images show no elongation and are symmetric. Their size is equivalent to the beam size for the reconstruction process.

In addition to our speckle results, other observations of SN1987A show strong evidence for an asymmetrically expanding shell<sup>6</sup>. Early spectroscopic observations (between 20 and 80 days after the explosion) detected a double-peaked feature in several hydrogen lines at optical and infrared wavelengths<sup>7–8</sup>. The appearance of two equally displaced components at longer and shorter wavelengths than the maximum emission was interpreted as being due to some departure from spherical symmetry of the shell.<sup>10</sup> Polarimetric observations<sup>11–14</sup> also show strong evidence for asymmetry in the envelope with a position angle of  $\sim 200^\circ$ . The observed polarization structure was interpreted as arising from an asymmetrically expanding, scattering atmosphere. Polarimetric data were modelled by a scattering atmo-

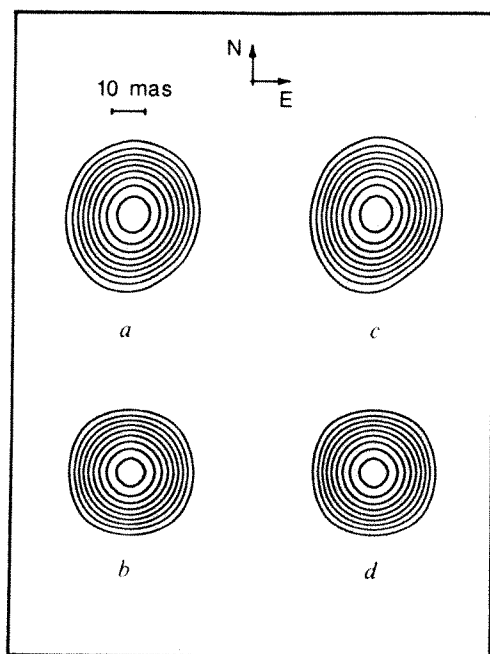


FIG. 2 Image reconstructions of SN1987A and a comparison star recorded in April 1988. *a*, SN1987A at 533.0 nm. *b*, Comparison star at 533.0 nm. *c*, SN1987A at H $\alpha$ . *d*, Comparison star at H $\alpha$ .

TABLE 1 Measured asymmetries for SN1987A (PA =  $200 \pm 5^\circ$ )

Days after explosion	$\lambda$ (nm)	Average diameter (mas)	Major axis (mas)	Minor axis (mas)
95–98 (May–June 87)	5,330	$18 \pm 2$	$19 \pm 2$	$17 \pm 2$
	6,565 (H $\alpha$ )	$8 \pm 1$	$10 \pm 2$	$6 \pm 2$
	7,750	$15 \pm 2$	$20 \pm 2$	$10 \pm 2$
265–268 (Nov. 87)	6,585 (H $\alpha$ )	$17 \pm 1$	$18 \pm 3$	$14 \pm 3$
	8,500	$16 \pm 1$	$20 \pm 2$	$14 \pm 2$
370–373 (Feb.–Mar. 88)	4,420	$25 \pm 2$	$27 \pm 2$	$22 \pm 2$
	5,330	$20 \pm 1$	$24 \pm 2$	$14 \pm 2$
	6,400	$17 \pm 1$	$20 \pm 2$	$15 \pm 2$
	6,565 (H $\alpha$ )	$21 \pm 1$	$24 \pm 2$	$16 \pm 2$
	7,000	$17 \pm 1$	$20 \pm 2$	$16 \pm 2$
	8,500	$18 \pm 2$	$25 \pm 2$	$12 \pm 2$
409–411 (April 88)	5,330	$26 \pm 2$	$30 \pm 2$	$20 \pm 2$
	6,565 (H $\alpha$ )	$27 \pm 1$	$30 \pm 2$	$20 \pm 2$
	8,500	$24 \pm 2$	$28 \pm 3$	$16 \pm 3$

sphere shaped as a prolate or oblate spheroid<sup>12,13,15</sup>. Best fits have been obtained for ratios of the smallest to the largest axis of these spheroids ranging from 0.6 to 0.9. Recently,  $\gamma$ -ray observations have yielded linewidths requiring either fragmentation or asymmetry of the supernova shell for their explanation<sup>16</sup>.

Present results from speckle interferometry, polarimetry, spectroscopy and  $\gamma$ -ray observations show that the expanding atmosphere of SN1987A is not spherically symmetric. The theoretical models that assume spherical symmetry must clearly be revised. An asymmetric envelope may also be necessary to explain, for example, the early emergence of X-rays and  $\gamma$ -rays and the evolution of their spectra with time<sup>17</sup>. Woosley<sup>18</sup> has suggested that non-uniformities in the collapse and core bounce could produce an asymmetric explosion, but that this asymmetry is far less likely to propagate to the outer envelope. Chevalier and Soker<sup>19</sup> have modelled the expanding supernova envelope and concluded that the most likely mechanism for asymmetry is rotational flattening of the progenitor envelope, probably requiring a binary companion during stellar evolution. Much of the evidence for asymmetries and extended structures around the supernova is summarized by Trimble<sup>20</sup>.  $\square$

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# Deuterium content of the Venus atmosphere

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THERE is no liquid water on Venus. The water vapour in its atmosphere would, if condensed, form a layer only 20 cm deep which means, in contrast to the 3-km-deep oceans that cover its sister planet Earth, that Venus is very dry indeed. It is not known with certainty whether Venus has lacked water since its formation, or if water once present has been lost during its lifetime; the question is of special interest as water is generally thought to be a necessary ingredient for the development of life. The abundance of deuterium in the atmosphere of Venus is an important clue to the planet's history, because ordinary and deuterated water escape at different rates. Using the high-resolution mode of the International Ultraviolet Explorer (IUE), we measured hydrogen Lyman- $\alpha$ -emission but found only an upper limit on deuterium Lyman- $\alpha$ -emission, from which we inferred a D/H ratio of less than  $2\text{--}5 \times 10^{-3}$ . This is smaller by a factor of 3–8 than the D/H ratio derived from measurements by the Pioneer Venus Large Probe, and may indicate either a stratification of D/H ratio with altitude or a smaller overall ratio than previously thought.

The Pioneer mass spectrometer detected a signal at mass 19 (HDO<sup>+</sup>), when the inlet became clogged by a droplet of sulphuric acid. The detection was taken<sup>1</sup> to imply a D/H ratio of  $1.6 \times 10^{-2}$ . If this modern ratio derives from an original D/H ratio similar to Earth's, that is,  $1.6 \times 10^{-4}$ , in sea water, but enriched by differential evaporation, Venus would have had enough water to form an ocean 20 m deep, although this is an extreme lower limit, because some deuterium would undoubtedly have escaped too.

The presence of HDO in the atmosphere of Venus will produce some D atoms by photodissociation and some consequent D Lyman- $\alpha$ -emission in the planet's ultraviolet airglow. In the upper atmosphere, the Lyman  $\alpha$  transition of H and D atoms can be excited by resonance scattering of solar photons, which can be observed by remote sensing from outside. The emission lines are separated by 0.33 Å (H emission is at 1,215.66 Å, and D at 1,215.33 Å), which is much more than the thermal width ( $\sim 10^{-2}$  Å) but less than the solar linewidth, so that both lines can be excited to an approximately equal extent.

The D Lyman- $\alpha$ -emission has been measured previously<sup>2</sup> in the upper atmosphere of the Earth with a dedicated Ly $\alpha$  spectrometer on board Spacelab 1, and was found to have an intensity of 300 rayleigh along a tangential line of sight at an altitude of 110 km, just above the O<sub>2</sub> absorption level. The corresponding D/H ratio was found to be  $\sim 3 \times 10^{-4}$ , indicating a twofold enrichment in D relative to sea water which results from the smaller exospheric escape for D atoms than for H atoms.

The International Ultraviolet Explorer (IUE), launched in 1978, has in principle the spectral resolution required to separate both D and H Ly $\alpha$  lines, and it was therefore proposed that it be used to detect deuterium in the upper atmosphere of Venus at the  $\sim 1\%$  level for D/H ratio following the Pioneer mass spectrometer measurement of HDO in the lower atmosphere.

A series of high-dispersion SWP (short-wavelength primary camera) spectra of the Venus dayside disk has been obtained with IUE, both in the course of this programme and in others. Measurements of the IUE echelle-grating scattered-light profile have shown that scattered H Ly emission 0.33 Å from the line centre—that is, where D Ly $\alpha$  emission should occur—is well below the level of  $10^{-2}$  expected for such emission<sup>3</sup>, so that the

detection of D Ly $\alpha$  becomes a problem of signal-to-noise ratio. In principle, the small aperture of IUE (circular, 3.2") would give the best separation of D Ly $\alpha$  and H Ly $\alpha$  spectral lines. However, the large aperture (23 × 10") has a throughput on an extended source that is greater than that of the small aperture by a factor 30, and it was found that large-aperture SWP spectra of the longest duration (that is, with the H Ly $\alpha$  emission feature saturated) provided the best measurement of D Ly $\alpha$ , despite the slight overlap of the D and H features, because of the extended size of the aperture. D emission should be apparent as an asymmetric lineshape (specifically, as a short-wavelength shoulder; see Fig. 1).

Some of the archived spectra were taken with only part of the aperture imaging the disk of Venus, leading to a distorted Ly $\alpha$  lineshape. The photowrite images of all spectra were therefore examined so as to select only those images for which the aperture was filled. A customized reduction method was required to analyse these spectra, because the aperture width used to sum the emission region in the standard IUE reduction is designed to separate the orders of the echelle and is too narrow to include all of the emission entering the large aperture. The Venus spectra contain discrete Ly $\alpha$  emission with no adjacent continuum other than longer-wavelength light scattered by the cross-disperser grating across the region of Ly $\alpha$ . In the customized reduction we therefore (1) summed the entire emission region of the Ly $\alpha$  lines; (2) employed a more extended region of background measurement for an accurate removal of the scattered light; (3) re-processed all images with VAX floating-point accuracy in the intensity transfer functions using the SDPS processing routines at Goddard Space Flight Center; and (4) carefully aligned all spectra in wavelength to the observed centre of the H Ly $\alpha$  line before summing. Thermal drifts of the spectrum across the detector face with time are advantageous, as the fixed noise pattern of the SEC cameras is altered when the images are realigned in wavelength and the noise level is therefore reduced in the summed spectrum.

The longest archived exposures of Venus lasted for 60 min: exposures longer than this were precluded by extreme saturation of the camera at the long-wavelength end of the spectrum. Six spectra ranging in duration from 30 to 60 min were summed as described and the result is plotted in Fig. 1. These spectra were obtained near the Venus maximum elongation, on 12 and 13 April and 26 August 1980. The Ly $\alpha$  intensity, estimated from other shorter, unsaturated exposures, was found to be

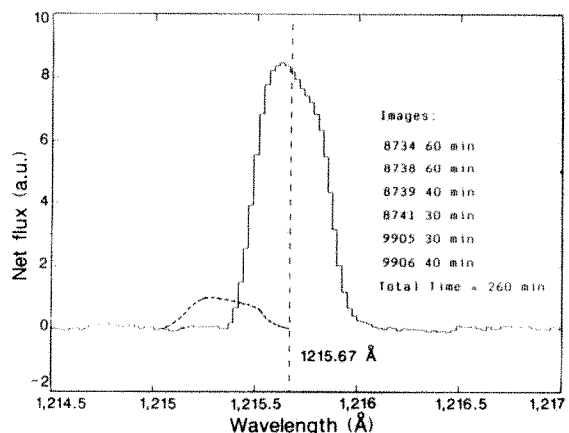


FIG. 1 Hydrogen Ly $\alpha$  spectra of bright-disk emission from Venus, obtained with the IUE large aperture and summing the six best images (solid line). The line intensity corresponds to  $21 \times 10^3$  rayleigh; the long-dashed line shows the line centre. The short-dashed line shows the profile calculated for deuterium Ly $\alpha$  emission of  $2.5 \times 10^3$  rayleigh (corresponding to a D/H ratio of  $1.6 \times 10^{-2}$ ). On the basis of the IUE observations, we can rule out such emission down to a factor of  $\sim 8$  times less than this. The numbers refer to spectra reference numbers and exposure times. a.u. denotes  $10^5$  IUE flux units.

$(21 \pm 5) \times 10^3$  rayleigh, which is consistent with the Pioneer Venus Orbiter ultraviolet spectrometer (PVO UVS) observations<sup>4,5</sup>. Although there is some saturation for the images summed in Fig. 1, the IUE procedure is able to account for this moderate saturation because intensity for the average spectrum is the same as that found for unsaturated exposures. The dashed line in Fig. 1 shows D Ly $\alpha$  emission of  $2.5 \times 10^3$  rayleigh modelled by scaling down the observed spectrum by a factor of 8 and displacing it by 0.33 Å. As such a shoulder does not appear in the observed spectrum, there seems to be no significant D Ly $\alpha$  emission; an examination of the noise level in this region allows us to place an upper limit ( $2\sigma$ ) of 300 rayleigh for such an emission. Although this implies an upper limit of 1.5% on the ratio of intensities, we shall see below that it implies a substantially lower D/H density ratio.

In the upper atmosphere of Venus, D and H atoms are excited by the solar Lyman  $\alpha$  line at about the same rate. However, CO<sub>2</sub> absorbs Lyman  $\alpha$  at an altitude of 110 km, and only those atoms above this level contribute to the emerging intensity. The IUE upper limit ( $2\sigma$ ) of  $I_D = 300$  rayleigh implies an upper limit for the vertical column density,  $N_D$ , of D atoms above 110 km of  $N_D = I_D \times 10^6 / g_d$ , where  $g_d$  is the excitation rate of D atoms. The ratio  $g_d/g_h$  (with  $g_h$  defined in similar fashion for H atoms) can be estimated from the relative flux intensities at the solar line centre,  $F_s$  (H excitation), and at D resonance (displaced from line centre by 0.33 Å); these measurements have been made by OSO-5 (ref. 6) (Orbiting Solar Observatory), and indicate a ratio of 0.8. The value of  $g_h$  may be found from  $g_h = 0.544 \times 10^{-14} F_s s^{-1}$ , with  $F_s = 8 \times 10^{11}$  photon  $cm^{-2} s^{-1} \text{Å}^{-1}$  at Venus (ref. 4). Variations in  $F_s$  over the 2-year period of IUE observations were smaller than  $\pm 10\%$  (ref. 5), this being confirmed by monitoring of the total solar Ly $\alpha$  flux from Earth's orbit by SME. Thus,  $g_h = 4.35 \times 10^{-3} s^{-1}$ , so that  $g_d = 3.5 \times 10^{-3} s^{-1}$  and, for  $I_D = 300$  rayleigh, we obtain  $N_D = 8.6 \times 10^{10}$  atom  $cm^{-2}$ . The photometric uncertainty of IUE at the Ly $\alpha$  wavelength is estimated as  $< 25\%$ ; indeed, the H Ly $\alpha$  intensity of  $21 \times 10^3$  rayleigh measured by IUE agrees well with the nadir, subsolar value of  $20 \times 10^3$  rayleigh measured by PVO UVS at the same time<sup>4,5</sup>.

The ratio  $I_D/I_H$  from the disk of Venus is different from the D/H density ratio in the lower atmosphere, mainly because the atmosphere above 110 km is optically thin at Ly $\alpha$  for D atoms but is moderately thick for H atoms, so that here  $I_D/I_H$  is larger than the ratio of column densities  $N_D/N_H$ . Because of the nonlinear dependence of  $I_H$  on  $N_H$ , one cannot derive  $N_H$  directly from these IUE observations. Thus to estimate  $N_H$  we use instead the Pioneer observations analysed by Paxton *et al.*<sup>4,5</sup>; these were dayside observations, made not far from the subsolar point, and therefore are pertinent to the present IUE observations.

The PVO UVS was better situated than IUE to determine an absolute value of the H density in the Venus atmosphere, because the Pioneer measurements were performed both from outside and from inside the H atmosphere, and also because the spinning geometry of PVO UVS permits measurement of bright disk intensities as well as limb profiles. The H medium is optically moderately thick so that the shape of the Ly $\alpha$  variation over one spacecraft spin depends upon the absolute H concentration everywhere between 110 km and the outside exosphere. The H density vertical distribution can therefore be determined without knowledge of either the Ly $\alpha$  centre solar flux or the UVS calibration<sup>4</sup>, by comparing radiative transfer predictions, made on the basis of a wide range of models of H density distribution, with the observations.

Paxton *et al.*<sup>5</sup> use two parameters to describe the H vertical profile: the exobase density  $n_e$  at 200 km, and the escape flux  $\phi_e$  (also at 200 km), which, for a given  $n_e$ , influences the density at the homopause level. The PVO UVS results indicate that, over the two-year period of observations (May 1979 to May 1981), the H profile near the subsolar point is remarkably con-

stant and can be described by  $n_e = (6 \pm 1.5) \times 10^4 \text{ cm}^{-3}$  and  $\phi_e = 7.5 \pm 1.5 \times 10^7 \text{ cm}^{-2} s^{-1}$ . The corresponding column density  $N_H$  above 110 km was determined to be  $(2.4 \pm 0.8) \times 10^{13} \text{ cm}^{-2}$  (ref. 5).

Combining the various uncertainties quadratically gives an upper limit ( $2\sigma$ ) to the ratio  $N_D/N_H$  of  $(3.6 \pm 1.5) \times 10^{-3}$ . The ratio of densities  $n_D/n_H$  at some reference level (say, 100 km) will be slightly different from the value of  $N_D/N_H$ , because the D and H vertical profiles are not identical. Integrating the equation of diffusion<sup>7</sup> for D atoms using the VIRA atmospheric profile<sup>8</sup> we obtain the relation  $n_D/n_H(100 \text{ km}) = \alpha (N_D/N_H)$ . If the escape flux of D atoms is zero then  $\alpha = 0.55$ ; if it is equal to the H escape flux (relative to the exobase density at 200 km) then  $\alpha \approx 1$ , and if it is 0.32 to make a rough allowance for the mass fractionation effect, then  $\alpha \approx 0.97$ .

Therefore, regardless of the value assumed for the D escape rate,  $N_D/N_H$  above 110 km is not very much different from the D/H ratio below the homopause. This is to be expected, as the homopause occurs at around 130 km well above the penetration level of Ly $\alpha$  in CO<sub>2</sub>. The upper limit of D/H at 100 km of  $\sim (3.6 \pm 1.5) \times 10^{-3}$  is lower by a factor of 4.4 than the value previously reported for the lower atmosphere<sup>1</sup>, and in fact is probably closer to  $2 \times 10^{-3}$  if present-day deuterium escape is negligible, which is entirely possible.

This low value can be compared with another set of measurements made by the Pioneer Venus Orbiter ion-mass spectrometer. Ions of mass 2 were originally identified as H<sub>2</sub><sup>+</sup> ions,<sup>9</sup> but were later assigned to D<sup>+</sup> (refs 10, 11). A detailed ionospheric model<sup>12</sup> of the pre-dawn bulge ionosphere supported this assignment to D<sup>+</sup> but showed that it required D/H neutral-atom ratios at the homopause of 1.4 and 2.5% respectively for the two orbits during which measurements were made. Clearly, the non-detection of D Ly $\alpha$  emission in the IUE data argues against such a quantity of deuterium at the homopause, suggesting that the mass-2 ions may indeed have been H<sub>2</sub><sup>+</sup> ions. To obtain an H<sub>2</sub><sup>+</sup> signal of the magnitude observed would require an H<sub>2</sub> abundance of 10 p.p.m. on Venus<sup>13</sup>, which is consistent with previous gas-chromatograph data from Venera 13-14 (ref. 14).

Despite the apparently small content of deuterium in the upper atmosphere of Venus, it is possible that more substantial amounts exist in the lower atmosphere—as suggested by the identification of a mass-19 signal, measured by the Neutral Mass Spectrometer, with HDO<sup>+</sup>—as a result of some atmospheric fractionation process. On the other hand, Spacelab-1 results have shown clearly that such processes do not operate on the Earth, and given that the aeronomy of H<sub>2</sub>O, H<sub>2</sub> and H does not seem to differ greatly for the two planets, they would not be expected to occur on Venus either. It seems possible, therefore, that the D/H ratio measured in the upper atmosphere of Venus does indeed represent the bulk atmospheric ratio. The Hubble Space Telescope will provide the means to test these results, as its High Resolution Spectrometer will be capable of resolving a D/H ratio of  $1.6 \times 10^{-4}$  (the value on the Earth) on Venus. □

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# Tripolar vortices in a rotating fluid

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THE emergence of coherent flow structures is a well-known feature of quasi-geostrophic or two-dimensional turbulence, and because of their relevance to large-scale geophysical flows the dynamics of these structures have been studied increasingly over the past decade<sup>1-5</sup>. The most common coherent structures are the axisymmetric (monopolar) vortex, with circular streamlines, and the vortex dipole, both of which have been found to arise in a variety of situations under different (sometimes nondescript) forcing conditions. The emergence of vortex dipoles, for example, has been observed in turbulence in soap films<sup>3</sup>, in electrically forced magnetohydrodynamic flows<sup>5</sup> and in collapsing turbulence in a continuously stratified fluid<sup>6</sup>. There is growing evidence<sup>4,7-9</sup> that, in addition to the monopolar and dipolar vortices, a tripolar coherent flow structure exists. Here we describe a laboratory experiment in which a tripolar structure is found to emerge from an unstable cyclonic vortex in a homogeneous rotating fluid. The tripole seems to be a very stable structure, even persisting in a highly sheared fluid environment, and might therefore be found in natural geophysical flows.

Our experiment is part of a larger study of the dynamics of barotropic vortices in a rotating fluid<sup>10</sup>. The laboratory set-up (Fig. 1) consists of a cylindrical perspex tank (92.5 cm in diameter and 30 cm deep) mounted concentrically on a turntable, whose angular speed is continuously adjustable from 0 to 10 r.p.m. The tank is filled with a homogeneous fluid, and after the table has been set in steady motion, the fluid is spun-up to solid-body rotation. For these experiments the spin-up time-scale is typically 40 times the rotation period of the turntable, and the Ekman number based on the water depth is small, typically  $\sim 10^{-5}$ . Both cyclonic and anticyclonic vortices were created by briefly stirring the fluid confined in a bottomless cylinder placed at the centre of the tank. After allowing the stirring-induced flow to become a purely azimuthal flow, the inner cylinder is withdrawn vertically, releasing the vortex into the solidly rotating ambient fluid. In most experiments the Rossby number associated with the initial vortex flow—based on the vortex diameter and the maximum velocity—was large, that is, of order unity. The resulting motions were recorded photographically by a remote-controlled co-rotating camera mounted above the free surface. The flow of the initially confined fluid was traced by the addition of dye, and streak photography of small tracer particles floating on the free surface provided quantitative information on the total velocity field. Because of the two-dimensionality of the flow, the motion at the free surface, as measured from the streak lengths, is representative of the flow at lower levels.

The evolution of a cyclonic stirring-induced barotropic vortex is illustrated by the sequence of photographs shown in Fig. 2. Immediately after releasing the vortex by lifting the inner cylinder, turbulent mixing is observed in the highly sheared region at the circumference of the vortex (Fig. 2a). Within a few rotation periods, however, the flow settles in an approximately circular vortex with a smoother edge. A slightly non-axisymmetric internal structure becomes established in the dyed fluid (Fig. 2b, c), and eventually the vortex exhibits a tripolar structure, with cyclonic motion in the core and anticyclonic motion concentrated in the two satellite vortices (Fig. 2d, e, f). The tripole structure rotates in a solid-body-like manner around its centre, in a cyclonic direction relative to the rotating frame, as can be seen in the successive photographs. The motion in the interior of the tripole decays slowly, as does the overall rotation speed.

This decay is caused partly by bottom friction and partly by lateral entrainment of initially quiescent ambient fluid into the satellite vortices (visible in Fig. 2d and f). Initially (that is, just after release of the cyclonic stirring-induced vortex, Fig. 2a) anticyclonic vorticity is confined to a narrow region around the core of the cyclonic vortex. The presence of this vorticity can be inferred from the shearing of the dye filaments that get wrapped around the central structure (Fig. 2b). The anticyclonic vorticity gradually becomes concentrated in the two anticyclonic satellite vortices, an effect associated with the growth of a perturbation of azimuthal wavenumber  $m = 2$ . In many stability studies (for example, refs 11 and 12) this is found to be the fastest-growing mode for unstable circularly symmetric vorticity distributions.

In some additional experiments barotropic vortices were generated in a different way, by taking the fluid level inside the inner cylinder (Fig. 1) lower than that outside. When the cylinder is lifted, gravitational collapse results in an axisymmetric swirling motion, as would be expected from conservation of angular momentum. Although the flow immediately after the collapse is very turbulent, the motion becomes purely azimuthal typically within two rotation periods. These experiments<sup>10</sup> revealed that the vortices remain axisymmetric for relatively large values of  $\bar{H}$  and/or  $\Delta H/\bar{H}$  ( $\bar{H}$  being the mean water depth and  $\Delta H$  the difference in level), whereas a gradual transition towards a tripole structure was observed for relatively small values of both  $\bar{H}$  and  $\Delta H/\bar{H}$ . For these collapse-induced cyclonic vortices the timescale associated with the tripole formation is considerably longer than for those created by the stirring technique. This is probably a consequence of the different vorticity distributions of each type of vortex: the ring of anticyclonic vorticity encompassing the cyclonic-vorticity core is much wider for the collapse-induced vortices than for the vortices induced by stirring.

Quantitative measurements of the velocity field associated with the tripolar vortex were obtained by streak photography of tracer particles floating on the free surface; a typical example is shown in Fig. 3. Such measurements allow calculation of the spatial distribution of the vorticity  $\omega$  and the streamfunction  $\Psi$ ; the relationship between these quantities—when measured in a reference frame co-moving with the tripole—provides important information about the tripole dynamics. Although in many theoretical studies<sup>13-16</sup> of (monopolar and dipolar) coherent flow structures a linear relationship between  $\omega$  and  $\Psi$  is assumed, laboratory experiments<sup>5</sup> on magnetohydrodynamic vortex dipoles indicate the existence of a nonlinear relationship. Our preliminary laboratory measurements<sup>8</sup> of the digitized velocity field of tripole structures also suggest nonlinearity in the  $(\omega, \Psi)$  relationship; further details will be published elsewhere. The information provided by digitized streak photographs should be essential for a theoretical description of tripoles.

Although in a 'real' tripole the vorticity is distributed continuously over a compact region consisting of three aligned

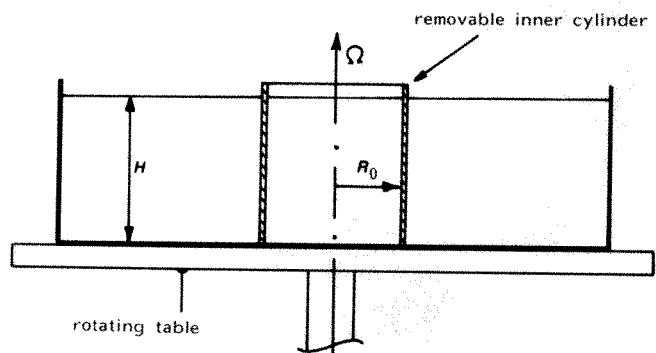


FIG. 1 A schematic drawing of the laboratory set-up used to produce barotropic vortices in a rotating fluid.



patches (the two satellites and the central region, containing anticyclonic and cyclonic vorticity respectively), we can model a tripole crudely in terms of three equidistantly aligned point vortices with circulations of  $-1$ ,  $2$  and  $-1$ , respectively. Such an idealized configuration of vortices exhibits a steady cyclonic rotation around the central vortex, as observed for the laboratory tripoles. The streamfunction of the flow associated with a rotating point-vortex tripole relative to the rotating frame has been calculated<sup>8</sup>, and the corresponding pattern of streamlines (Fig.

4) shows good agreement with the dye patterns observed in the laboratory (Fig. 2*d* to *f*). But because no information about, say, the relation of the circulation of the laboratory tripole to its overall rotation rate is available yet, the quantitative value of such a point-vortex model is not clear at present.

Motivated by their interaction properties, it has been speculated<sup>17,18</sup> that isolated vortices, that is, monopolar and dipolar structures, are the 'particles' of geophysical fluid dynamics, in the sense that, although interacting, they are able to maintain

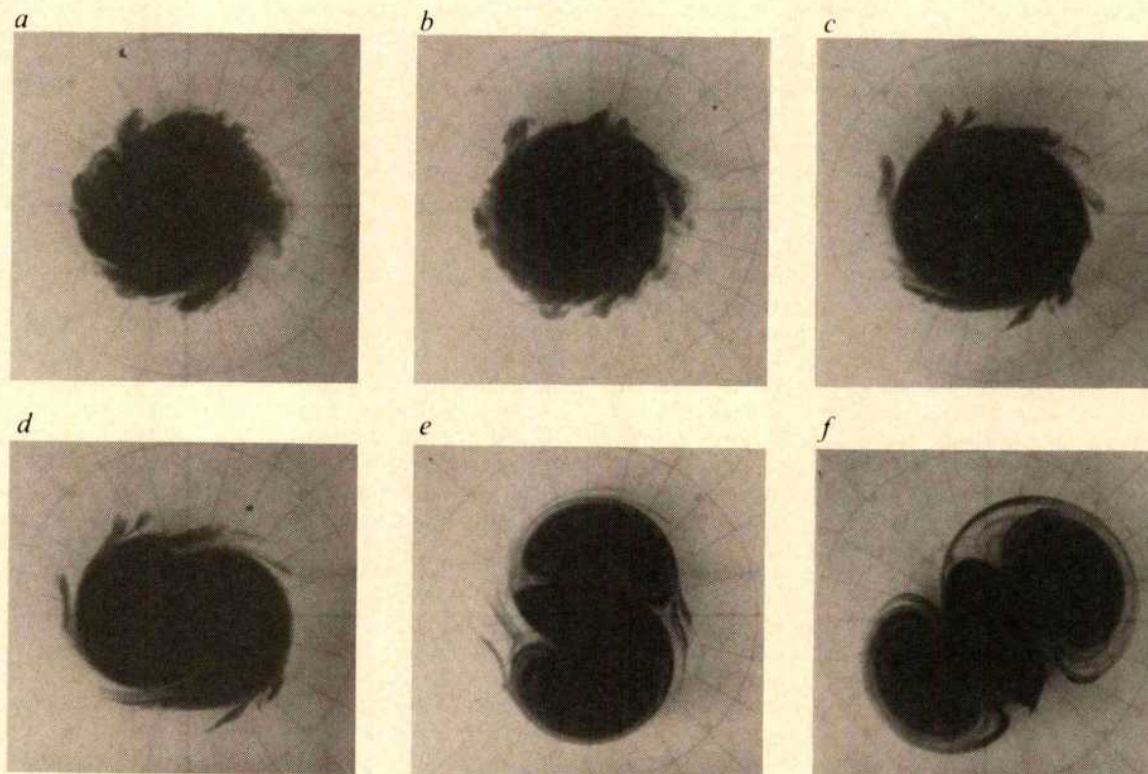


FIG. 2 A sequence of plan-view photographs illustrating the emergence of a tripolar flow structure from a cyclonic barotropic stirring-induced vortex. The photographs were taken at times of *a*,  $1.2 T$ , *b*,  $2.4 T$ , *c*,  $7.1 T$ , *d*,  $8.9 T$ ,

*e*,  $10.7 T$  and *f*,  $23.8 T$  after releasing the vortex, with  $T=8.4$  s, the rotation period of the turntable. The mean water depth,  $H$ , was  $14.5$  cm, and the initial vortex diameter,  $2R_0$ , was  $11.0$  cm.



FIG. 3 A typical streak-line photograph of the relative flow field associated with a slowly rotating tripolar vortex.

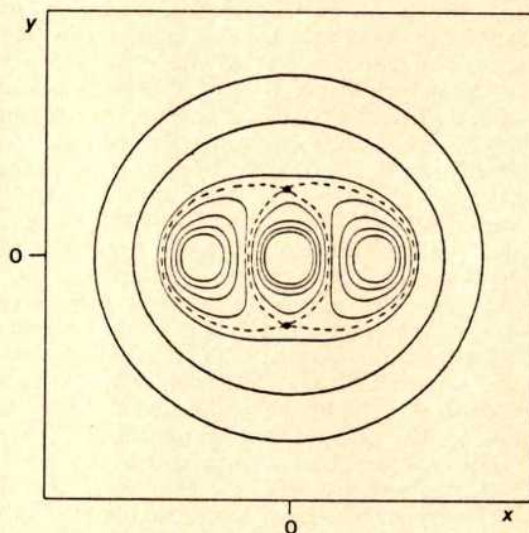


FIG. 4 Theoretical streamline pattern of a rotating tripolar vortex modelled<sup>8</sup> as three equidistantly aligned point vortices with circulations  $-1$ ,  $2$  and  $-1$ .

their structural identity much better than can dispersing waves. Monopoles and dipoles can be considered as basic flow structures in two-dimensional flows; the former are stationary features containing net angular momentum, whereas dipoles are steadily translating vortex structures that contain net linear momentum. The tripolar vortex, as found in these laboratory experiments and also in numerical simulations of two-dimensional turbulence<sup>4</sup>, appears to be an equally basic flow structure, characterized by a net angular momentum and a steady rotation of the structure as a whole. Its observed persistence in sheared fluid environments suggests that the tripole may be a robust and stable vortex structure. Tripoles have not yet been observed in large-scale geophysical flows, but in view of this apparent stability it is not unlikely that oceanic examples will be found in the near future. □

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## Compositional convection in viscous melts

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**DURING** solidification of multi-component melts, gradients in temperature and composition develop on different scales because of the large difference between their respective molecular diffusivities. Two consequences are the development of double-diffusive convection<sup>1</sup> and the creation of mushy zones in which solid and liquid intimately coexist with a complex small-scale geometry<sup>2,3</sup>. Theoretical analysis requires simplifying assumptions that must be verified by laboratory experiments. Hitherto, experiments have been carried out with aqueous solutions which do not accurately represent the dynamics of melts with high Prandtl numbers, such as magmas. Here we describe the characteristics of compositional convection using a new experimental technique which allows the viscosity of the solution to be varied independently of chemical composition and liquidus temperature. A super-eutectic melt was cooled from below, causing the growth of a horizontal layer of crystals. Convective instability occurred when the local solutal Rayleigh number of the compositional boundary layer ahead of the advancing crystallization front attained a value of  $\sim 3$  on average. We observed a novel regime of convection in which the thermal boundary layer above the crystallization front was essentially unmodified by the motion of the plumes. The plumes carried a small heat flux and did not mix the fluid to a uniform temperature.

We have carried out experiments with aqueous solutions of ammonium chloride ( $\text{NH}_4\text{Cl}$ ) plus small amounts of

hydroxyethylcellulose compound. These low concentrations ( $< 2 \text{ wt}\%$ ) of the latter do not affect the phase diagram but nevertheless allow the solution viscosity to be varied by several orders of magnitude (Fig. 1). The solution was placed in the experimental tank and maintained at a uniform temperature above the liquidus. The base plate of the tank was connected to a pre-cooled bath at time  $t=0$ , and reached a constant temperature (below the liquidus) after a short transient. Temperatures in the fluid were measured with a vertical array of thermocouples. A few minutes after cooling began, small crystals of  $\text{NH}_4\text{Cl}$  were observed, distributed randomly at many places across the base. Growth continued by the lateral expansion of crystal patches and new nucleation until a mushy layer with a horizontal upper surface became established. Figure 2 shows the thickness of the layer as a function of time.

Our solutions contained concentrations of  $\text{NH}_4\text{Cl}$  greater than that of the eutectic point and hence fractional crystallization produced a boundary layer of light residual liquid. Compositional convection was eventually observed in the form of a field of plumes rising from the crystallizing region (Fig. 3a). The plumes consisted of cylindrical conduits and rounded caps, similar to 'diapir' plumes<sup>4</sup>. Plume cap diameters were in the range 0.3–1.0 cm, that is, substantially larger than the crystal size ( $< 0.1 \text{ cm}$ ), and increased with solution viscosity. An intriguing feature of previous experiments with ammonium chloride solutions is the formation of so-called 'chimneys'<sup>5</sup>. These are vertical channels in the mush through which strong upward flow of residual melt occurs, fed by lateral flow through the porous crystal network. Chimney formation was not observed in our experiments—we always observed the continuous generation of new starting plumes across the whole area of the tank. This is more consistent with the instability of a boundary layer at the top of the mush, analogous to the intermittent release of thermals from a diffusive boundary layer in high-Rayleigh-number thermal convection<sup>6</sup>. It may be that at higher solution viscosities chimneys develop only at times longer than the total duration of our experiments or that lateral flow in the mush is suppressed. Increasing the cooling rate at fixed viscosity resulted in a systematic increase of the critical time for the onset of convection, in agreement with previous qualitative observations<sup>5,7</sup>. Furthermore, we have observed how the critical time varies with solution viscosity (Fig. 3b).

Theory shows that convective instability of a chemical boundary layer above a flat crystal face advancing at constant velocity<sup>8,9</sup> occurs when a local Rayleigh number, defined using the boundary layer thickness ( $d$ ) as a length scale, exceeds a critical

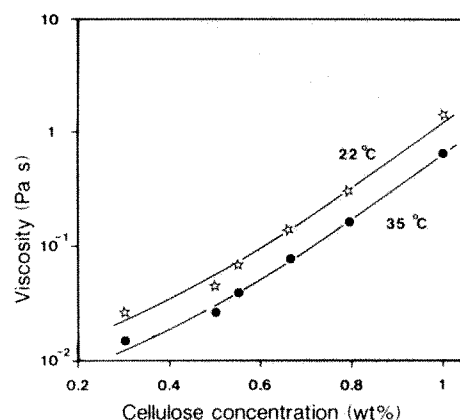


FIG. 1 The viscosity, determined with a falling-ball viscosimeter, of 28 wt%  $\text{NH}_4\text{Cl}$  solution as a function of the concentration of cellulose additive. The approximate initial temperature of the solution was 35 °C (lower curve), and the typical temperature measured at the crystallization front was 22 °C (upper curve). Viscosities from the latter curve were used in calculations on the instability of the compositional boundary layer.



value  $Ra_c$  given by

$$\frac{g\Delta\rho_c}{D\mu}d^3 = Ra_c \quad (1)$$

where  $\Delta\rho_c$  is the compositional density difference across the boundary layer,  $g$  is the acceleration due to gravity,  $D$  is the diffusivity of the chemical species involved and  $\mu$  is the solution viscosity. In this analysis,  $d$  is given by  $D/V$ , where  $V$  is the fixed rate of advance of the crystal face.

In our experiments, constitutional supercooling occurs and, instead of a flat crystal-liquid interface, a mush forms<sup>3,10</sup>. Until the onset of compositional convection, theory predicts that temperature and composition evolve by diffusion in a regime for which the interface advances as  $2\lambda\sqrt{Dt}$ . The constant  $\lambda$  depends on the liquidus slope, the latent heat, the thermal and chemical diffusivities, and the cooling conditions<sup>2,3</sup>. In this theory, crystallization starts instantaneously at time  $t=0$ , whereas in our experiments some time is required for nucleation and the establishment of a horizontal crystal layer (Fig. 2). Some non-zero value of initial time must be adopted in order to fit a  $\sqrt{t}$  law, and times rather longer than the duration of our experiments are required to accurately verify this law. Depending on the initial temperature contrast, we obtain values of  $\lambda$  ranging from 3 to 6, which are close to those found using the theoretical framework of Worster<sup>3</sup>.

The compositional profile in the boundary layer above the mush is<sup>3</sup>:

$$C(z, t) = \Delta C \frac{\text{erfc}(z/2\sqrt{Dt})}{\text{erfc}(\lambda)} + C_i \quad (2)$$

where  $C_i$  is the initial liquid composition,  $\Delta C$  is the compositional difference across the boundary layer and  $\text{erfc}$  is the complementary error function<sup>11</sup>. Given the interface temperature and the assumption of local thermodynamic equilibrium,  $\Delta C$  was estimated for this set of experiments to be  $\sim 0.1$  wt%  $\text{NH}_4\text{Cl}$ . A rough check of this estimate was made by measuring the contrast in electrical conductivity of the solution across the boundary layer. The boundary layer thickness is defined as

$$d = \frac{\Delta C}{\left(\frac{\partial C}{\partial z}\right)} = \sqrt{\pi Dt} \exp(\lambda^2) \text{erfc}(\lambda) \quad (3)$$

which is of the form  $d = \alpha(Dt)^{1/2}$ . As  $\lambda \rightarrow \infty$ , equation (3) reduces to  $d = D/V$ , where the instantaneous velocity  $V = \lambda(D/t)^{1/2}$ . For  $\lambda > 3$ , as in our experiments, the difference between these expressions is less than 10%. Using the length scale  $D/V$  in equation (1) yields an expression for the critical time for the onset of convection,  $t_c$ :

$$t_c = \left( \frac{\lambda^3 Ra_c \mu}{g \Delta\rho_c \sqrt{D}} \right)^{2/3} \quad (4)$$

Our data support this viscosity scaling (Fig. 3b), which suggests that the essential physics of the situation is the same as for the case of a flat crystal face. Using  $D = 2.3 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$  (ref. 12) and  $\Delta\rho_c = 1 \text{ kg m}^{-3}$ ,  $Ra_c$  lies between 1 and 8, with an average value of 3, which is at the lower end of the range of theoretical results for a flat crystal face<sup>9</sup>. This suggests that fluid from deep within the mush does not participate in the initial instability. Within this framework, plume diameter should scale with the thickness of the unstable boundary layer, which is also consistent with our data.

Thermal diffusion acts over a much greater thickness of fluid than merely the compositional boundary layer above the mush. The thermal field is such as to produce a stable density gradient but the compositional field is destabilizing. Theory predicts that until the onset of compositional convection, the temperature of

the mush-liquid interface is constant<sup>3</sup>. Figure 4 shows temperature profiles in the lower part of the tank, in two different experiments, recorded as the mush-liquid interface became level with each thermocouple in turn. The viscosities of the solution at the advancing interface were 1.4 Pa s (Fig. 4a) and 0.045 Pa s (Fig. 4b). In the first case (Fig. 4a) no convection was observed. The temperature of the mush-liquid interface is essentially constant, as expected. For long times, however, it exhibits a slight decrease, probably because our tank is of finite dimensions and hence the far-field temperature does not remain constant. In the latter case (Fig. 4b) compositional convection started after 27 min and continued throughout the experiment.

The main result is that a thick thermal boundary layer is observed despite the presence of compositional convection. The heat flux carried by the plumes is small, and manifests itself by slight quantitative differences in the evolution of the temperature profiles (Fig. 4) and the rates of advance of the mush-liquid interface in the convecting and conducting cases (Fig. 2). The plumes are thin and rise slowly, equilibrating with the surroundings by thermal diffusion over a time that is short compared with their rise time. Thus they are associated with very small temperature fluctuations ( $< 0.05^\circ\text{C}$ ), as we have verified by a series of detailed continuous temperature measurements. This can be understood by considering the ratio of the buoyancy in the compositional boundary layer to that in the thermal boundary layer ( $[\Delta\rho_c/\Delta\rho_T]\sqrt{D/\kappa}$ , where  $\kappa$  is the thermal diffusivity of the solution). If this ratio has a value  $\gg 1$ , the plumes have enough buoyancy to thoroughly mix the thermal boundary layer into the overlying fluid. A ratio  $\ll 1$  implies that the thermal stratification will be essentially undisturbed. The value of this ratio is  $\sim 0.05$  in our experiments, and is  $\sim 0.005$  for basaltic magmas. We conclude that, in this regime, the heat budget is dominated by conductive transfer.

This regime of compositional convection bears some resemblance to the 'salt-finger' regime of double-diffusive convection<sup>13</sup>. In salt-finger convection, the overall density gradient is stable and vertical motion is driven by horizontal diffusion of heat. Parcels of fluid retain their compositional difference with the surrounding fluid, but the vertical temperature gradient is unperturbed by the motion. In our experiments, the overall density gradient is unstable. Because the plumes are thin and sluggish, substantial lateral thermal diffusion occurs as they rise.

Our experiments illustrate that compositional convection involves two timescales, one characteristic of crystal growth and one characteristic of convective instability. Because the composi-

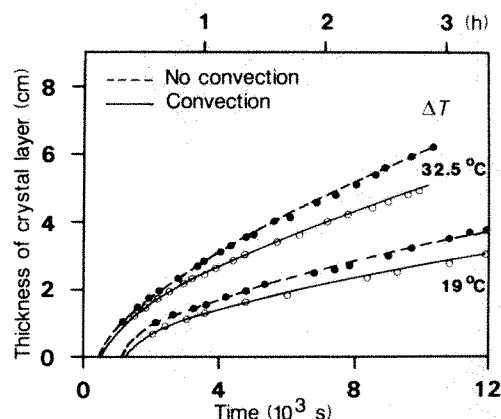


FIG. 2 The rate of growth of the crystal layer.  $\Delta T$  is the temperature difference between the base plate and the initial temperature of the solution. Dashed curves are for experiments in which  $\mu = 1.4 \text{ Pa s}$  and no convection occurred. Solid curves are for experiments in which  $\mu = 0.045 \text{ Pa s}$  and abundant convection occurred. Each pair of a dashed and a solid curve show the difference in the advance of the interface for experiments with the same thermal conditions. Differences are small, indicating that compositional convection makes a small contribution to the heat budget.



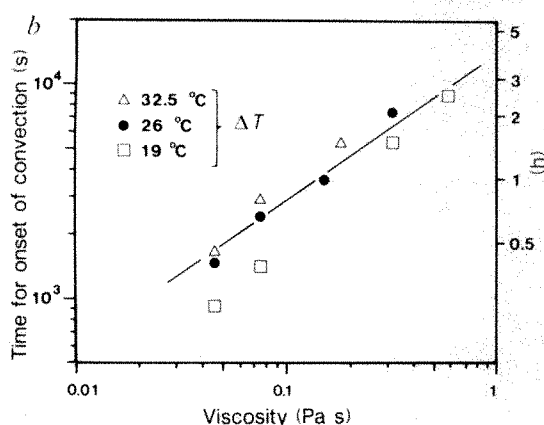
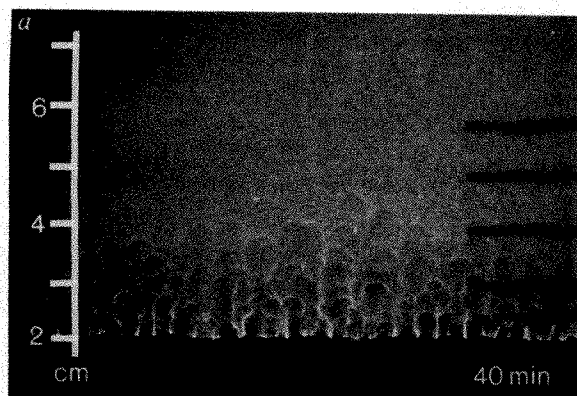


FIG. 3 *a*, Photograph showing the development of crystallization and compositional convection at the base of the tank ( $\mu = 0.045$  Pa s). *b*, Measurements of the critical time ( $t_c$ ) for the onset of convection as a function of solution viscosity.  $\Delta T$  is the temperature difference between the base plate and the initial temperature of the solution.  $t_c$  increases systematically with cooling rate. The data are also consistent with the prediction based on a local Rayleigh-number instability criterion for the boundary layer that  $t_c$  scales with solution viscosity to the power  $2/3$  (solid line). This line is drawn for  $Ra_c = 3$ .

tional boundary layer itself develops by diffusion during crystal growth, it is not possible for the latter to be much shorter than the former. Approximate equality of the two timescales corresponds to the case of aqueous solutions. The other possibility, crystal growth being more rapid than the development of convective instability, may arise naturally in, for example, igneous intrusions.

In mafic magma, growth of olivine and pyroxene releases less dense residual melt and thus, by analogy with our experiments, compositional convection can occur. At the onset of convection, the vertical flux into the crystallizing region of the compatible chemical components is increased, which should result in an increase in the modal proportions of these minerals in the crystallized product. Although large undercoolings can be attained, the propagation of the crystallization front during crystallization of magma always occurs at a rate proportional to  $\sqrt{t}$  (ref. 14). We assume that compositional convection begins when the local solutal Rayleigh number exceeds a value of 3. From equation (1), the critical value of the mush-liquid interface velocity below which convection can occur,  $V_c$ , is

$$V_c = \left( \frac{g \Delta \rho_c D^2}{\mu Ra_c} \right)^{1/3} \quad (5)$$

For an illustrative calculation for basaltic magmas, we take  $\mu = 10$  Pa s,  $D = 10^{-12}$  m<sup>2</sup> s<sup>-1</sup> (values corresponding to Mg<sup>2+</sup> and Fe<sup>2+</sup> in mafic melts<sup>15</sup>) and  $\Delta \rho_c = 10$  kg m<sup>-3</sup> (ref. 16), for which equation (5) gives  $V_c = 1.5 \times 10^{-8}$  m s<sup>-1</sup>, that is, a few tens of centimetres per year.

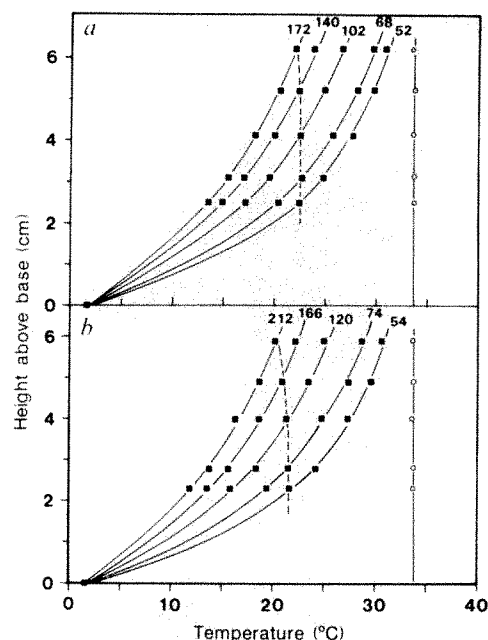


FIG. 4 Vertical profiles of temperature in the lower part of the tank, revealing the structure of the double-diffusive boundary layer generated by crystallization. Open circles show the initial uniform temperature of the solutions. The numbers give the time in minutes at which profiles were recorded. Dashed lines show the crystallization-front temperature, solid line segments to the right of the dashed lines show the profiles above the front, and those to the left show the profiles in the mush. In both experiments, the solution has a liquidus temperature of 24 °C and the base plate temperature was 1.5 °C. *a*,  $\mu = 1.4$  Pa s,  $\Delta T = 32.5$  °C. No convection occurred, and temperature evolved by conduction alone. *b*,  $\mu = 0.045$  Pa s,  $\Delta T = 32.5$  °C. Despite abundant compositional convection, there is a thick thermal boundary layer above the front—see discussion in text.

A question of practical importance to petrologists is: what thickness of rock will have crystallized by the time compositional convection starts? The crystallization front  $X(t)$  moves according to  $X = 2\lambda^* \sqrt{\kappa t}$  (ref. 14) and thus the front velocity is  $V = \lambda^* \sqrt{\kappa/t}$ . Hence

$$X(t) = 2\lambda^* \kappa / V \quad (6)$$

Using the above value of critical velocity and taking  $\kappa = 7 \times 10^{-7}$  m<sup>2</sup> s<sup>-1</sup> and  $\lambda^* = 1$  (ref. 14), we find that  $\sim 90$  m of rock would crystallize before the onset of convection. Clearly there is a range in the values of the governing parameters for natural magmas, and here we intend to obtain only an order-of-magnitude estimate.

In mafic sills, a layer with increased modal olivine content is commonly observed at a certain distance from the lower contact. The height above the lower contact of olivine concentrations for several sills of different overall thicknesses and related to different magmatic episodes ranges from 10 to 20 m (refs 17–20). In large mafic intrusions, there is typically a basal sequence overlain by thick olivine cumulates. In the Stillwater Complex, North America<sup>21</sup>, the Muskox Intrusion, Canada<sup>22</sup> and the Great 'Dyke', Zimbabwe<sup>23</sup>, these basal sequences are observed to be about 100 m thick. The above thicknesses are all of roughly the same order of magnitude, suggesting that the appearance of mineralogical layering results from local phenomena. We propose that this may be due to the onset of compositional convection, because the thicknesses are broadly similar to the result obtained above from equation (6). This hypothesis can be verified by further petrological studies.  $\square$

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## Contamination of Indian Ocean asthenosphere by the Kerguelen–Heard mantle plume

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THE mid-ocean-ridge basalts (MORBs) from the Indian Ocean are isotopically distinctive. They have higher  $^{87}\text{Sr}/^{86}\text{Sr}$ ,  $^{208}\text{Pb}/^{206}\text{Pb}$  and  $^{207}\text{Pb}/^{206}\text{Pb}$  ratios than their Pacific and Atlantic counterparts<sup>1,2</sup> (see Fig. 1), suggesting that the upper mantle beneath the Indian Ocean has remained a partially isolated system<sup>2</sup>. The Australian–Antarctic discordance, a bathymetrically depressed segment of the South-East Indian Ridge, may represent a fundamental boundary between the Indian and Pacific mantle domains<sup>3</sup>. What processes are responsible for such domains? We argue here that the Kerguelen–Heard plume, which is producing Dupal-type basalts on Kerguelen and Heard Islands in the southern Indian Ocean, has contaminated large volumes of the Indian Ocean asthenosphere. This has produced the distinctive composition of Indian Ocean MORBs.

Figure 2 illustrates the tectonic evolution of the southern Indian Ocean, and the development of tectono-magmatic features that may be attributable to the Kerguelen–Heard plume. The diagrams suggest that the plume has been involved in the development of several major features of the Indian Ocean lithosphere, and indicate that it has been active during the past 115 Myr. Lower Cretaceous continental magmatism in south-west Australia (Bunbury Basalts: 105–136 Myr BP<sup>4</sup>), north-east India (Rajmahal Traps: 108–128 Myr<sup>5</sup> and lamprophyres: 105–121 Myr<sup>6</sup>) and Antarctica (Prince Charles Mountains lamprophyres: 108–110 Myr<sup>7</sup>) imply that the plume was active during, and perhaps before, the separation of India from Antarctica–Australia at ~122 Myr<sup>8</sup>.

Major oceanic structures that can be attributed to the Kerguelen–Heard plume are the Kerguelen Plateau, emplaced during the Lower Cretaceous<sup>9</sup>, possibly at a ridge setting

analogous to that of Iceland today; and the Ninetyeast Ridge, a 5,000-km-long aseismic ridge<sup>10</sup>. Both features testify to huge volumes of magma being erupted throughout much of the Cretaceous and Tertiary. But do the chemical data for these features support the contention that they were all derived from the Kerguelen–Heard Plume? And do the data support the hypothesis that the same plume has contaminated large volumes of the Indian Ocean asthenosphere?

Kerguelen Island has been magmatically active for at least the past 40 Myr<sup>11</sup>. An important feature of Kerguelen Island magmatic activity is the changing isotopic and trace-element compositions with time; the younger basalts have higher  $\epsilon_{\text{Sr}}$  and lower  $\epsilon_{\text{Nd}}$  (refs 12, 13). During its early history, the island (and the hotspot) was probably adjacent to the embryonic South-East Indian Ridge. Mixing between plume mantle and either MORB asthenosphere or sub-plateau lithosphere diluted the effect of the plume<sup>12</sup>. Thus, the younger basalts may be isotopically more representative of the plume mantle, for example:  $^{87}\text{Sr}/^{86}\text{Sr} \approx 0.7051$ – $0.7059$ ;  $\epsilon_{\text{Nd}} \approx -1.7$  to  $-5.7$ ;  $^{208}\text{Pb}/^{204}\text{Pb} \approx 38.5$ . These isotope data are typical of Dupal-type ocean island basalts<sup>14</sup>.

Basalts from Kerguelen and Heard Islands form an array with approximately constant La/Th ratios (Fig. 3). The elements La, Ta and Th are highly incompatible in basaltic systems and ratios of these elements probably reflect the compositions of the mantle source; certainly, most ocean islands plot in distinct fields on this diagram<sup>15</sup>. Such systematic variations cannot be easily accommodated by fractionation during magma genesis. The Kerguelen and Heard array overlaps the field for Gough Island, an island in the South Atlantic with a strong Dupal component<sup>16</sup>, although some Heard Island samples have lower Th/Ta and La/Ta ratios.

In Pb-isotope space, two analysed tholeiites from the Ninetyeast Ridge<sup>1</sup> overlap the field of Kerguelen–Heard basalts (Fig. 1), suggesting a Dupal-like character. In terms of certain trace-element ratios (for example, Zr/Nb ratios<sup>17</sup>), the tholeiites are intermediate between Kerguelen Island plume basalts and MORBs. There is considerable scatter on the Th/Ta–La/Ta diagram, however, and many of the samples do not fall on such a mixing line (Fig. 3).

A suite of dredged tholeiites from the 77° graben<sup>9</sup>, in the southern part of the Kerguelen Plateau, has been analysed for Pb, Sr and Nd isotopes and major and trace elements. These results will be published elsewhere. The basalts are characterized by high  $^{207}\text{Pb}/^{204}\text{Pb}$  and  $^{208}\text{Pb}/^{204}\text{Pb}$  ratios, overlapping with the data from the Ninetyeast Ridge, the South-East Indian Ridge

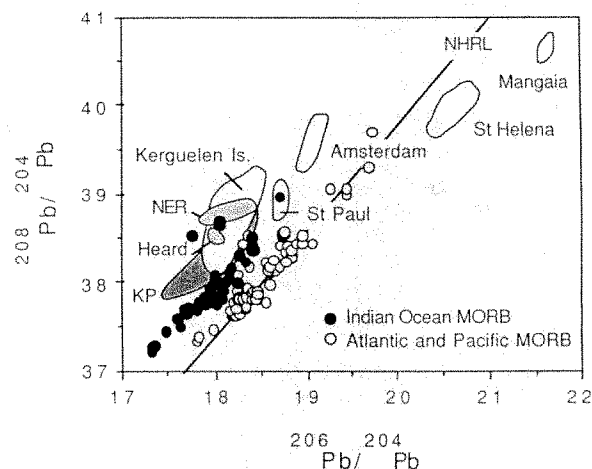


FIG. 1  $^{208}\text{Pb}/^{204}\text{Pb}$  against  $^{206}\text{Pb}/^{204}\text{Pb}$  for Indian, Atlantic and Pacific oceanic basalts. Data sources: Atlantic and Pacific MORB, refs 24–26; Indian MORB, refs 24–27 (and references in ref. 12); Kerguelen Plateau (KP), ref. 18; Kerguelen and Heard Island, ref. 12; Ninetyeast Ridge (NER), ref. 1; St Paul, refs 1 and 2; Amsterdam, ref. 1; St Helena, ref. 16; Mangaia, ref. 29; Northern Hemisphere Reference Line (NHRL), ref. 14.

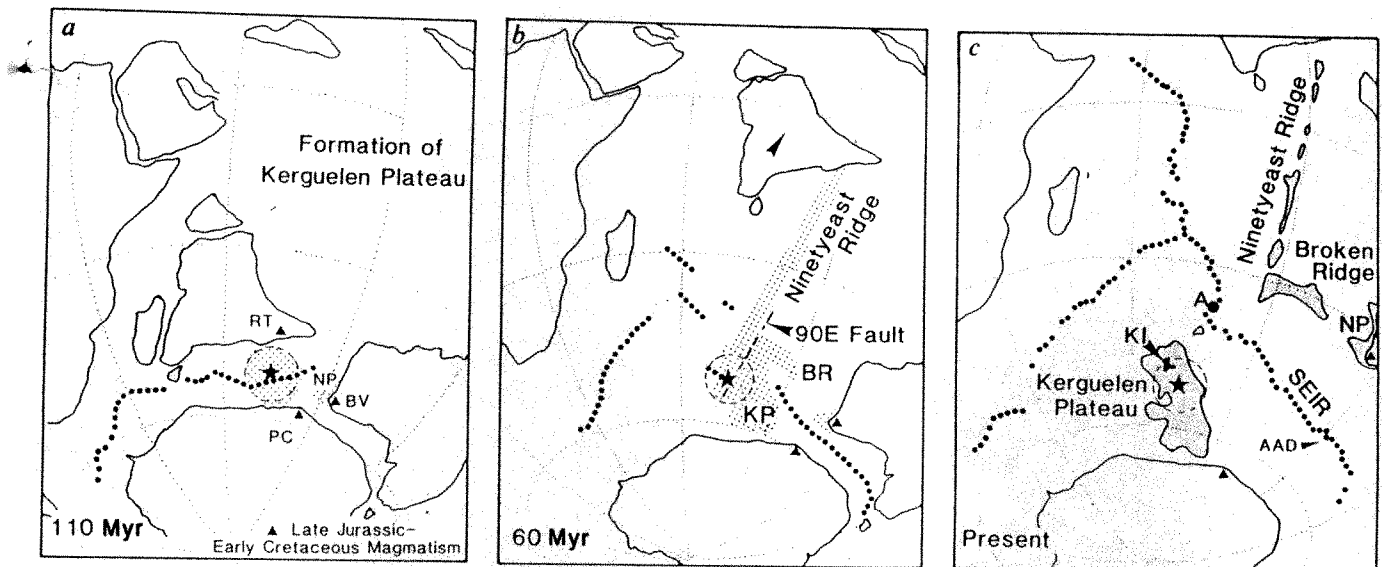


FIG. 2 Schematic maps to illustrate the evolution of the southern Indian Ocean, and the interactions of the Kerguelen-Hearde plume (KHP) with various plate boundaries. The reconstructions were created using the computer package 'Terra Mobilis' (C. Denham & C. Scotese, Geoimages, Texas). The plume (with a 1,000-km-diameter head) is assumed to have a fixed coordinate beneath the present-day location of Heard Island (star). KI, Kerguelen

Island. BV, Bunbury Volcanics (ref. 4); RT, Rajmahal Traps and lamprophyres (refs 5 and 6); NP, Naturaliste Plateau; PC, Prince Charles Mountains lamprophyres (ref. 7); SEIR, South-East Indian Ridge; AAD, Australia-Antarctic Discordance (ref. 3); A, Amsterdam and St Paul Islands. Stippled ornament represents regions of thickened oceanic lithosphere.

MORBs, and the Kerguelen Archipelago (Fig. 1)<sup>18</sup>. A striking feature of the Kerguelen Plateau data is the range of Th/Ta and La/Ta ratios of the dredged basalts (Fig. 3), perhaps indicating an extreme expression of the Dupal component observed in Kerguelen Island basalts<sup>12</sup>. The extent to which the high Th/Ta ratios observed in the plateau basalts are representative of the plateau as a whole must await further data from basalts recovered during Leg 120 of the Ocean Drilling Program. We note here that high Th/Ta ratios are found in some continental flood basalts<sup>19</sup> and lamprophyres<sup>20</sup> (Fig. 3), and they are probably a feature of the continental lithosphere (crust or sub-continental mantle). The high Th/Ta ratios found in four analysed Kerguelen Plateau basalts cannot be taken to indicate that the plateau is underlain by subcontinental lithosphere. However, one interpretation is that the sub-Gondwana lithosphere, with high Th/Ta ratios, was activated and stripped by the Kerguelen-Hearde plume and incorporated in the sub-Gondwana asthenosphere (Fig. 4), and then melted out during the formation of the Kerguelen Plateau lithosphere. Alteration of the basalts to produce high Th/Ta and La/Ta ratios cannot be completely excluded but we note that all three elements have low mobility during the low-temperature alteration which has affected these samples.

The possibility arises that the long-lived, vigorous Dupal Kerguelen-Hearde plume is responsible for the Dupal-like character of modern Indian Ocean MORBs, through pervasive contamination of the sub-oceanic asthenosphere. Hamelin *et al.*<sup>2</sup> have suggested that mixing between MORB asthenosphere and plume mantle is responsible for generating the distinctive Indian MORB isotopic arrays. To fit the data, at least two stages of mixing are required: (1) an earlier contaminant (the Kerguelen-Hearde plume in this paper) which was responsible for the broad elevation in  $^{208}\text{Pb}/^{206}\text{Pb}$  (and  $^{87}\text{Sr}/^{86}\text{Sr}$ ) ratios, and which affected large volumes of the Indian Ocean asthenosphere; and (2) subsequent mixing between this contaminated asthenosphere and other high- $^{206}\text{Pb}/^{204}\text{Pb}$  mantle plumes in the central Indian Ocean (for example, St Paul and Amsterdam islands). This second event appears to be responsible for the spread to high  $^{206}\text{Pb}/^{204}\text{Pb}$  values on Pb-Pb isotope plots (Fig. 1). The La/Ta-Th/Ta data are broadly consistent with mixing between depleted MORB mantle (DMM), with a high

La/Ta ratio<sup>15</sup>, and the plume mantle. (Unfortunately, no suitable trace-element data are available for basalts from St Paul and Amsterdam islands.) However, none of the analysed South-East Indian Ridge basalts has high Th/Ta ratios. This suggests that mixing between a DMM and a high Th/Ta (lithospheric?) component was not an important process in the evolution of the sub-Indian Ocean MORB asthenosphere, although Th, La and Ta data are required from other Indian Ocean ridge segments to confirm this suggestion.

Evidence for plume contamination of adjacent sub-oceanic asthenosphere is preserved elsewhere; for example, Iceland<sup>21</sup>,

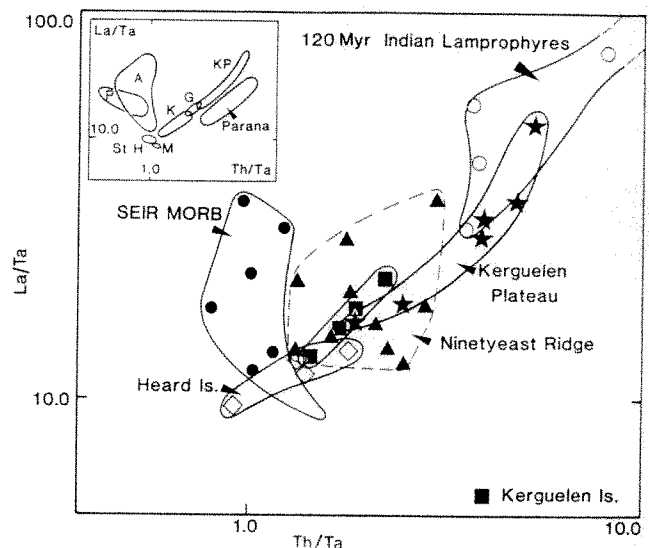


FIG. 3 La/Ta against Th/Ta for Indian Ocean basalts and (inset) other oceanic and continental (Parana) regions. Data sources: Heard and Kerguelen (K) Islands, ref. 12; SEIR MORB, ref. 30; Ninetyeast Ridge (DSDP Sites 214, 216, 253 and 254) and Kerguelen Plateau (KP), this study; Indian lamprophyres, ref. 20; Pacific (P) and Atlantic (A) MORBs are extrapolated from diagrams in ref. 15 (and references therein) assuming a Ce/La ratio of 3.0 and a Nb/Ta ratio of 17.65; Mangaia (M), ref. 28; Gough (G) and St Helena (St H), ref. 16; Parana continental flood basalts, ref. 19.



Easter<sup>22</sup> and Ascension<sup>23</sup> islands. However, in these examples the extent of contamination appears to be far less widespread than in the Indian Ocean. There may be several reasons for this. First, the Indian Ocean is a relatively compact, youthful ocean, and it originated from a locus at the Kerguelen-Heard plume. Second, the plume is very distinct from MORB in having a strong Dupal character. The component is thus more easily detected in the MORBs than are the Icelandic and Ascension signatures. Third, the plume has interacted with major spreading axes throughout its history, and therefore has had access to the Indian Ocean mantle system (the other main Dupal islands, Gough and Tristan da Cunha in the South Atlantic, have had relatively localized access to the Atlantic system); and finally, the plume appears to have been active for a long period of time, and there is strong evidence (mentioned above) that it reactivated the sub-continental lithosphere above the embryonic Indian Ocean asthenosphere. The plume could therefore have been actively contaminating the asthenosphere before the break-up of Gondwana.

Identification of mantle domains associated with specific mantle plumes can only be achieved by detailed dredging of the present-day ridge system. Klein *et al.*<sup>3</sup> have recently identified a potential boundary between two oceanic mantle domains,

which may represent the eastern influence of the Kerguelen plume system. There are too few data to identify the western limit of the influence of the KHP; the basalts of the South-West Indian Ridge appear to have a Walvis Ridge component<sup>2</sup>, indicating the influence of another Southern Hemisphere Dupal domain. The unknown factor is the extent to which many of the distinctive Dupal characteristics—in plumes or MORBs—result from long-term residence beneath the stable Gondwana supercontinent.

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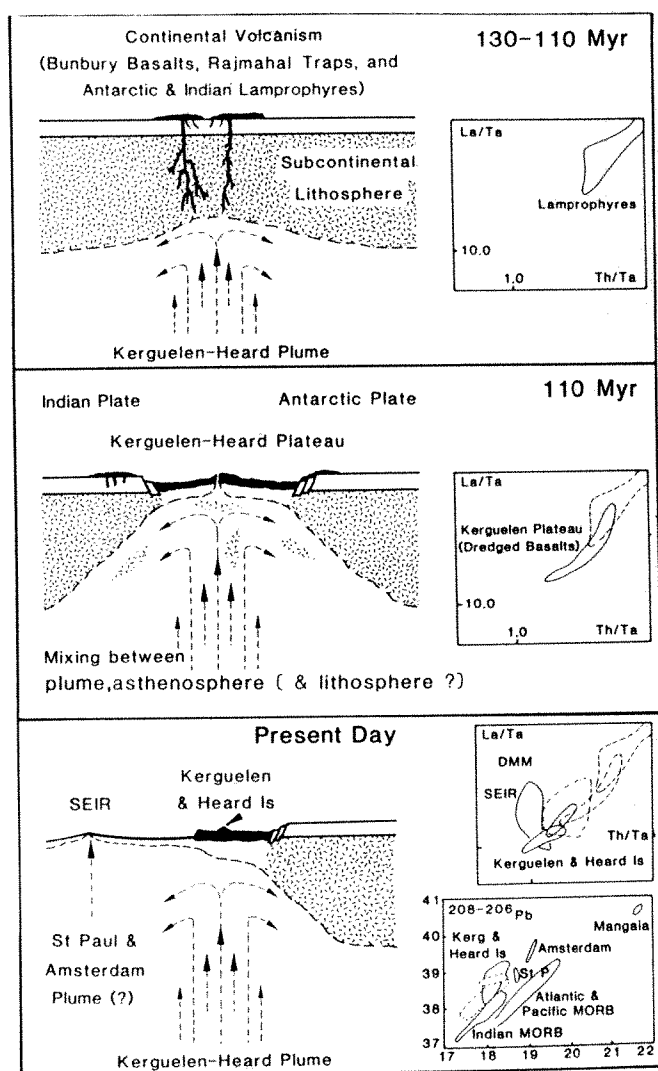


FIG. 4 Schematic diagrams to illustrate the interaction of the Kerguelen-Heard Plume with the sub-Gondwana lithosphere and with the sub-Indian Ocean asthenosphere. See text and Figs. 1–3 for discussion and data sources. DMM: Depleted MORB mantle (see ref. 15).

## Genetic specialists, kin recognition and nepotism in honey-bee colonies

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INSECT societies have long served as useful models in the study of two often intertwined issues in evolutionary biology: the levels at which natural selection operates<sup>1</sup>, and the phenomena of competition and cooperation in animal societies<sup>2</sup>. Hamilton<sup>3–5</sup> provided a darwinian explanation for the evolution of cooperation by invoking natural selection for increased inclusive fitness, whereas Trivers and Hare<sup>6</sup> showed how asymmetrical genetic relationships among members of hymenopteran societies result in differential fitness among queens and workers and can lead to conflict over the production of males. Here we provide evidence for competition among workers of the honey bee, *Apis mellifera*, in the production of female reproductives, a consequence of the genetic structure of colonies resulting from polyandry<sup>7</sup>, genotypic biases in components of cooperative behaviour associated with division of labour<sup>8–10</sup>, and kin recognition<sup>11–13</sup>. We also propose that nepotistic behaviour by honey bees is influenced by both kin- and colony-level selection.

Honey-bee colonies normally consist of about 17 subfamilies<sup>7</sup>. Members of the same subfamily, super sisters<sup>14</sup>, share both a

queen mother and a drone father and, in the absence of inbreeding, have a coefficient of genetic relationship of 0.75 (ref. 15). Half-sisters belong to different subfamilies, share only a mother, and have a genetic relationship of 0.25. We tested the ability of workers to discriminate during the rearing of queens in colonies that were derived from instrumentally inseminated queens and composed of three biochemically-distinct subfamilies. Samples of immature (larvae and prepupae) queens and workers, and adult workers located near either immature queens or workers, were collected from experimental colonies and analysed by protein electrophoresis to determine the frequencies of individuals belonging to each of the three subfamilies (see Fig. 1 and Table 1 for details).

Any observed subfamily biases in queen production could be a consequence of intracolony kin recognition coupled with one or more of four mechanisms: (1) workers preferentially raise the most familiar larvae, that is, those belonging to the subfamily with the highest larval frequency; (2) workers prefer-

entially raise the rarest larvae; (3) the subfamily with the most adult members on queen cells dominates queen rearing and preferentially raises super sisters; and (4) biases occur in the proportional representation of subfamilies engaged in the rearing of queens (relative to larval frequencies) and individuals preferentially rear super sisters. To determine which, if any, of these mechanisms are involved, four series of Monte Carlo simulations were conducted based on the results of our allozyme analyses. Each series of simulations focused on a different 'test'-subfamily that was appropriate to each one of these four possible mechanisms.

Our results demonstrate nepotism during the rearing of queens. Individual workers were located on or off queen cells, at least in part, on the basis of genotypically determined factors. Treatment of immature queens (involving perhaps differential feeding and/or selective removal) was apparently mediated by genetic relatedness with a small but consistent rearing preference shown for more closely related larvae. Subfamilies with a bias

TABLE 1 Nepotism in the rearing of queens by honey bees

Test-subfamily						Test-subfamily					
Total no. immature workers analysed	Total no. queens analysed	Weighted average of frequency in immature worker and queen samples	Expected no. queens reared	Observed no. queens reared	Deviation	Total no. immature workers analysed	Total no. queens analysed	Weighted average of frequency in immature worker and queen samples	Expected no. queens reared	Observed no. queens reared	Deviation
Colony 4438						Colony 4450					
49	29	0.46	12	15	+3	40	32	0.07	0	5	+5
50	28	0.37	10	11	+1	40	38	0.35	11	15	+4
39	7	0.33	2	3	+1	40	14	0.00	0	0	0
Colony 4439						Colony 4453					
40	6	0.35	2	3	+1	40	25	0.45	10	12	+2
40	24	0.38	10	8	-2	40	26	0.24	6	6	0
40	7	0.26	2	0	-2	40	17	0.28	5	3	-2
Colony 4440						Colony 4456					
50	33	0.30	9	11	+2	49	32	0.19	6	5	-1
41	44	0.39	13	21	+8	51	44	0.40	15	20	+5
40	28	0.31	8	9	+1	40	37	0.41	14	17	+3
Colony 4442						Colony 4457					
40	31	0.39	11	6	-5	40	36	0.38	10	18	+8
40	40	0.40	14	18	+4	40	40	0.55	19	25	+6
40	50	0.10	2	7	+5	40	53	0.28	15	15	0
Colony 4445						Colony 4464					
50	35	0.29	8	13	+5	40	29	0.06	1	2	+1
50	41	0.13	2	9	+7	40	36	0.04	1	2	+1
41	25	0.23	6	6	0	34	32	0.64	21	20	-1
Total deviation = +60											

The table demonstrates nepotism in the rearing of queens by honey bees based on kin recognition and subfamily differences in the likelihood of raising queens (mechanism 4 in text). In this case the 'test'-subfamily (one per trial for each colony, three trials total) was selected because it exhibited a higher probability of being located on queen cells than other subfamilies in the colony. For the analysis, four different sets of 'test'-subfamilies were selected, each set appropriate to one of the four hypothesized mechanisms underlying biases in the rearing of queens. To test the hypothesis of mechanism 4 we determined the relative likelihood of rearing queens,  $RL_s$ , for individuals of each subfamily ( $s$ ) within a colony:  $RL_s = PA_s / PI_s$ , where  $PA_s$  is the proportion of subfamily  $s$  in sample of adults on queen cells, and  $PI_s$  is the proportion of subfamily  $s$  in sample of immature workers. The subfamily with the highest value of  $RL$  for each colony in each trial was designated the 'test'-subfamily for mechanism 4. The expected number of queens for the test-subfamily  $E(Q_i)$  was then calculated from the equation  $E(Q_i) = (I_i/N)$ , where  $I_i$  is the proportion of test-subfamily in sample of immature workers, and  $N$  is the total number of queens raised in a given trial. The difference between  $E(Q_i)$  and the observed number of queens  $O(Q_i)$  was then calculated to determine the deviation. Because conventional analyses of contingency tables were inappropriate<sup>16</sup> (workers selected queens without replacement from among a finite sample of 60 transferred larvae), we used Monte Carlo simulations to estimate the probability of getting a deviation that was as great or greater than our result. For each of the four hypothesized mechanisms, the Monte Carlo simulation randomly drew a number of immature workers equal to the actual sample from a very large (infinite) pool with appropriate test-subfamily frequencies equal to the weighted average of the immature worker and queen samples. It then drew an independent random sample of immature queens from the same pool and calculated the deviation from expected as described above. Deviations were summed for all trials of all colonies. The simulation was iterated 1,000 times and iterations in which the total deviation was as great or greater than that observed were summed. The probability of getting a result as extreme as that observed was calculated as the number of iterations with a total deviation greater than that observed divided by 1,000. The entire simulation process was repeated five times to give an estimate of the standard error of the simulation-generated probability. Observed deviations cannot be a consequence of nonrandom sperm usage by queens because  $E(Q_i)$  and  $O(Q_i)$  were both based on the same samples of immature females.

in adult representation as queen nurses, relative to the available potential queen larvae, biased the colony's output of queens in their favour.

On average, only half ( $\bar{X} = 30 \pm 11.8$  s.d.; 916 queens were raised in total) of the 60 transferred larvae were raised during each trial of each colony, thus providing ample opportunity for workers to make 'choices' with respect to which larvae become queens. Combining data from all trials of all colonies, the subfamilies whose adult members had the highest within-trial individual probability of being sampled on queen cells (mechanism 4; Fig. 1) collectively had 60 more super-sister queens raised than expected on the basis of samples of immature workers ( $P = 0.001 \pm 0.0002$  s.e.,  $n = 5$  Monte Carlo simulations; see Table 1). In contrast, members of the numerically most common subfamily, again based on samples of immature workers (mechanism 1), had 37 fewer queens raised than expected. Individuals belonging to the rare subfamily (mechanism 2) had 13 more than expected ( $P = 0.179 \pm 0.0066$ ), and those belonging

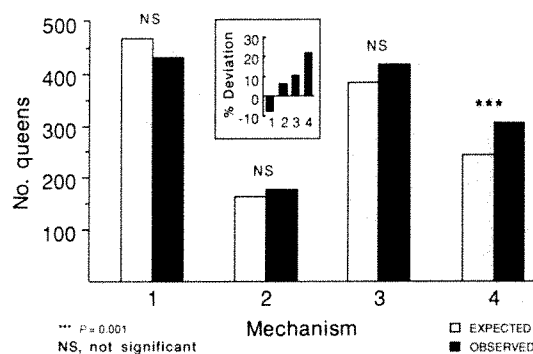


FIG. 1 Expected and observed number of queens reared for each of four mechanisms hypothesized to result (along with kin recognition) in nepotism in the rearing of queens by honey bees. The inset depicts the per cent deviation of the observed from the expected values for each mechanism. For explanation of statistical analyses see legend for Table 1.

**METHODS.** Ten colonies were established, each composed of three electrophoretically distinguishable subfamilies. The colonies' queens were daughters of three unrelated queen mothers. Drones for matings were progeny of eight additional unrelated queens. Each of the ten queen daughters was instrumentally inseminated<sup>23</sup> with semen from three unrelated drones, each one bearing a different malate dehydrogenase-1 (*Mdh*) marker allele, designated 'slow' (S), 'medium' (M) and 'fast' (F)<sup>24</sup>. Semen from each drone trio was pooled, diluted, and mixed before insemination to minimize temporal fluctuations in subfamily frequencies<sup>25,26</sup>. Four of ten inseminated queens were homozygotes at the *Mdh* locus (SS: colonies 4438, 4440, and 4456; MM: colony 4453), producing female progeny with three allozyme phenotypes that correspond to three subfamilies. Five queens (colonies 4439, 4442, 4445, 4450, and 4464) were SF heterozygotes that produced three subfamilies with five allozyme phenotypes: SS, SM, SF, MF, and FF. Larval and adult bees sampled from these colonies were grouped into three subfamilies as follows. SM and MF bees were assigned to the M subfamily because the M allele could only be paternally inherited. SF bees were assigned to the S and F subfamilies in proportion to the ratio of SS and FF phenotypes in each sample, because the maternal S and F alleles are expected to segregate in a 1:1 ratio. Subfamily membership was determined similarly for individuals from colony 4457, derived from a SM queen. The experiment took place from April to May, 1987, under conditions of seasonal production of queens associated with normal colony reproduction through fission<sup>27</sup>. At the start, nine of ten colonies (all except 4445) were raising queens naturally. Colonies occupied hives consisting of two or three chambers; the lower two were filled with combs of brood and honey, and the third with honey and empty combs. Each queen was confined to a bottom chamber of the hive with a queen excluder. For each colony, 60 newly hatched female larvae were transferred from a frame of worker comb containing up to 3,700 individual larvae into dry, artificial queen cells arranged 20 cells to a bar, three bars to a frame (female larvae located in special queen cells are fed differentially and develop into queens rather than workers<sup>28</sup>). The two frames were placed together in the centre of the upper brood chamber in each hive, with the queen cells closest to the side from which larvae were transferred. Three days later we removed the two frames, with minimal disturbance, and collected ~50 workers each from the surface of the queen cells and from the area of worker comb that originally contained the transferred larvae. These were assumed to be queen and worker 'nurses', respectively. Immatures (larvae and prepupae) were sampled six days after transfers when all investment in new queens was complete and the queen cells were capped by the workers<sup>28</sup>. In addition to collecting all queen cells, we gathered samples of about 50 immature workers that were the same age and located in the same area of the comb as were the queen larvae before they were transferred. All samples were stored at  $-70^{\circ}\text{C}$  until analysed by electrophoresis. Each colony was tested three times at 7–8-day intervals for a total of 30 trials. All queens, 34–51 immature workers, and 34–43 adult workers per sample were analysed.

to the subfamily with the highest frequency of adult workers on queen cells, regardless of numerical representation in the colony (mechanism 3), had an excess of 34 raised ( $P = 0.064 \pm 0.0034$ ). In 13 of the 18 trials that showed a positive bias in the rearing of queens, assuming mechanism 3, the numerically dominant adult subfamily was also the one whose members had the greatest bias in likelihood of being sampled as queen nurses and, therefore, were also classified as the test subfamily based on mechanism 4. In these cases, mechanisms 3 and 4 were confounding.

Genetic 'specialization' for rearing queens is suggested by the following results. In four of ten colonies, the subfamily distribution of adult workers sampled on queen cells differed significantly from the distribution of adult workers sampled on the adjacent comb containing worker larvae in at least one of three trials, even though the sampled frames were only ~2–4 cm apart ( $P < 0.05$ , colony 4440;  $P < 0.01$ , 4450;  $P < 0.01$ , 4456;  $P < 0.05$  and  $P < 0.01$ , 4464;  $G$  tests of heterogeneity<sup>16</sup>). In two colonies subfamily differences were significant for the three trials combined ( $P < 0.01$ , 4453 and  $P < 0.05$ , 4456). The number of trials that showed a significant deviation ( $P < 0.05$ ) was greater than that expected based on chance alone ( $P < 0.02$ ; binomial test, assuming 30 independent trials). These differences may be due either to paternally inherited behavioural traits that generate a bias in the representation of adult subfamily members on queen cells relative to their representation in the pool of potential queen zygotes, or, alternatively, to nonrandom sperm use by queens coupled with age differences between queen 'nurses' and worker 'nurses'. Results based on analyses of patterns of sperm use for each queen do not support the alternative explanation. The subfamily distribution in worker larvae samples fluctuated significantly among trials in only two of ten colonies ( $P < 0.01$ , 4445 and 4450), suggesting that the sperm of the three drones used for each insemination were well mixed in eight colonies.

Subfamily biases for the rearing of queens were small and consistent with previous studies. The ratio of the proportion of test-subfamily (assuming mechanism 4) adults sampled on queen cells to its proportion in the sample of worker larvae (see Table 1 legend) was  $2.3 \pm 1.90$  s.d. The mean ratio of the observed to the expected number of test-subfamily (mechanism 4) queens raised, based on the worker larval samples, was  $1.5 \pm 1.10$  s.d. Page and Erickson<sup>17</sup> reported a ratio of 1.6 in the number of queens reared when workers were given a choice of super-sister and less related ( $G = 0.25$  or  $0.31$ ) non-nestmate larvae. Visscher<sup>18</sup> found a bias of 1.5 in favour of a mixture of super- and half-sister nestmate eggs or larvae over unrelated non-nestmates. Noonan<sup>19</sup> demonstrated a bias of 1.2 in the visitation frequency of adult workers to queen cells containing super- versus half-sister larvae in colonies composed of two visually distinguishable subfamilies.

In agreement with previous studies<sup>8–10</sup>, our results suggest that the genotypes of workers affect colony organization by influencing the probability of performing behaviour associated with the division of labour. Worker genotype also results in differential social interactions, some of which may lead to intranidal conflict. Simply determining genetic relationships among colony members is not sufficient for testing kin-selection theory; the genetic relationships among interacting individuals must be considered (compare with ref. 20). Our results also demonstrate that kin selection is an important process in the evolution of social interactions involved in reproduction.

Larval kin recognition should increase the adaptive value of genotypes that result in a higher probability of the rearing of queens, driving them rapidly to fixation in populations. Perhaps this is a social analogue of meiotic drive<sup>21</sup> that distorts the proportional representation of queen genotypes (M.D. Breed, personal communication). Genetic variability and the rather modest levels of nepotism observed may be functionally linked and represent a compromise between levels of selection<sup>22</sup>. Kin selection may favour alleles that result in intracolony competition: high propensity to rear queens, better recognition, and



more discrimination. However, variation in the rearing of queens coupled with larval discrimination may reduce the reproductive output of colonies, a consequence of decreased rearing of queens due to adult-adult or adult-larva conflict, or a decrease in the ergonomic efficiency of the colony. If so, then colony-level selection may favour cooperation: a lower propensity for rearing of queens, and less discrimination. With additional genetic analyses of insect societies, the functional significance of the observed genetic variability, and the mechanisms underlying the intricate interplay of competition and cooperation may be further elucidated. □

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## Root lectin as a determinant of host-plant specificity in the *Rhizobium*-legume symbiosis

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**THE induction of nitrogen-fixing nodules in legume roots by soil bacteria from the genera *Rhizobium* and *Bradyrhizobium* is host-plant-specific. This specificity is expressed at an early stage of the infection process and results from multiple interactions between bacterial and plant products. Among these, it has been suggested that root lectin recognized by bacterial receptor molecules is an important determinant of host specificity<sup>1,2</sup>. Lectins are carbohydrate-binding proteins and it is known that legumes belonging to different cross-inoculation groups produce lectins with different sugar-binding specificities<sup>3</sup>. We have tested this suggestion by introducing the pea lectin gene into white clover roots using *Agrobacterium rhizogenes* as a vector. The 'hairy' clover roots that result can be nodulated by a *Rhizobium* strain usually specific for plants from the pea cross-inoculation group.**

*Rhizobium leguminosarum* bv. *viciae* nodulates the cross-inoculation group of pea, vetch and lentil, whereas clover is nodulated by *R. leguminosarum* bv. *trifolii*. The involvement of root lectin in determining this host-plant specificity was tested

by introducing the pea lectin gene (*psl*) into the roots of white clover and by studying the infection of transgenic clover roots by *R. leguminosarum* bv. *viciae*. Transgenic roots rather than transgenic plants were studied because of the problems of regenerating the latter.

We cloned *psl* into a binary vector<sup>4</sup> using *A. rhizogenes* LBA1334 (Ar1334) (ref. 5) to obtain transgenic clover roots. The *psl* gene encodes a glucose/mannose binding protein<sup>6,7</sup> and is expressed at low and developmentally controlled levels in pea roots during the plant's life cycle<sup>8</sup>. Pea lectin is localized at the surface of growing pea root hairs, which are the target cells for rhizobial infection<sup>9</sup>. Lectin-enhanced accumulation of infective rhizobia at pea root-hair tips correlates with increased infectivity of the rhizobial cells<sup>10</sup>.

Complete genomic *psl* (*psl* *lecA* provided by J. A. Gatehouse<sup>7</sup>) was cloned in the binary vector pBin19 (ref. 11). In addition, the full length *psl* complementary DNA<sup>12</sup> (provided by M. Stubbs) was cloned in the binary expression vector pAGS 35S HB (ref. 13) (provided by C. M. P. van Dun) between the plant viral 35S promoter and the nopaline synthase 3' end. These binary vectors are referred to as pBin19 *psl* and pAGS *cpsl*, respectively. (For details of the cloning steps see Fig. 1.)

The efficiencies of the binary vector systems were tested by determining the activity of a reporter gene product. Both vectors carry a neomycin phosphotransferase gene (NPTII, see Fig. 1) which can be expressed in transformed plant tissue. Hairy roots

TABLE 1 Nodulation of white clover hairy roots by *Rhizobium leguminosarum*

Agrobacterium strain and binary vector	No. nodulated plants			
	Inoculation with bv. <i>trifolii</i>			
	*10 d	23 d	30 d	40 d
LBA1334	13/20	18/20	20/20	20/20†
LBA1334 pBin19	7/15	14/15	15/15	15/15†
LBA1334 pBin19 <i>psl</i>	15/20	19/20	20/20	20/20†
LBA1334 pAGS <i>cpsl</i>	15/19	19/19	19/19	19/19†
	Inoculation with bv. <i>viciae</i>			
LBA1334	0/19	0/19	0/19	1/19‡
LBA1334 pBin19	0/15	0/15	0/15	0/15
LBA1334 pBin19 <i>psl</i>	0/20	5/20	8/20	14/20§
LBA1334 pAGS <i>cpsl</i>	0/19	2/19	6/19	11/19§

*Trifolium repens* L. (white clover) seedlings were grown with their roots on filter paper in Petri dishes containing Jensen-1% agar<sup>14</sup> supplemented with 0.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>. Transformation was achieved by superficially wounding the stems of 6–8-day-old seedlings 2 mm under the first pair of leaves with the tips of an electron microscope grid forceps (number 7, Dumont, Switzerland) carrying *A. rhizogenes*. Bacteria were grown on LC (ref. 24) plates containing full antibiotic concentrations (see Fig. 1 legend) for Ar1334, Ar1334 carrying pBin19 or Ar1334 harbouring pAGS *cpsl* or pBin19 *psl*. Following transformation, the main root was excised 2–3 mm under the wound and the first hairy roots emerged from the wound sites 4–5 days later in all plants. Seedlings were transferred to fresh nutrient plates 5–7 days after wounding and nodulation assays were performed as described previously<sup>14</sup>. For re-isolation of rhizobia, red nodules were surface-disinfected in 5% H<sub>2</sub>O<sub>2</sub> for 10 min, rinsed in sterilized water and crushed in 250 µl B-medium<sup>25</sup> and plated on B-medium<sup>25</sup>. Re-isolates from R/248 Rifr-induced nodules were rifampicin-resistant, in contrast to re-isolates from R/843-induced nodules. Colonies from re-isolated rhizobia were blotted to nitrocellulose sheets and allowed to react with monoclonal antibody (mAb) 3, specific for the O-antigen of R/248 (ref. 21). Only re-isolates from R/248 Rifr-induced nodules were recognized by mAb 3. Rhizobial cell envelopes were analysed by SDS-polyacrylamide gel electrophoresis as previously described<sup>26</sup>. A protein of relative molecular mass 45,000 and characteristic of R/248 Rifr (control) was present in bacteria re-isolated from R/248 Rifr-induced nodules and absent from re-isolates from R/843-induced nodules. Nodulation of *Vicia sativa* L. by R/248 Rifr-re-isolates and their failure to nodulate white clover roots confirmed that *R. leguminosarum* bv. *viciae* 248 Rifr nodulates hairy roots of white clover co-transformed with pBin19 *psl* or pAGS *cpsl*. White clover seeds of cultivar 'Dutch Clover' (6990.6300) were purchased from Kieft, Blokker, The Netherlands.

\* Days after inoculation with rhizobia.

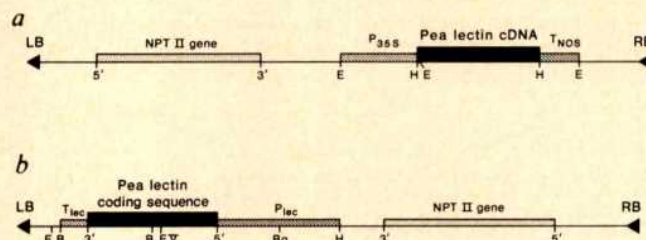
† Red nodules.

‡ Pseudonodules.

§ Red and white nodules and pseudonodules.



FIG. 1 Insertion of the pea lectin gene (*psl*) into binary vectors. To subclone the lectin cDNA as a *Hind*III fragment between the cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) polyadenylation signal of the binary vector pAGS HB 35S (a derivative of pAGS127) (ref. 13), *psl* cDNA (from plasmid pMS2) (ref. 12) coding for a segment of the lectin's leading signal and the complete  $\beta$ - and  $\alpha$ -chains, was first cloned into pIC-19H (ref. 7). The binary vector carrying the chimaeric pea lectin gene is pAGS *cpsl* (Fig. 1a). The complete genomic gene for *psl* (lecA) (ref. 7) was isolated as a *Hind*III–*Eco*RI restriction fragment and cloned into pBin19 (ref. 11). The resulting vector is termed pBin19 *psl* (Fig. 1b). Binary vectors were mobilized from *Escherichia coli* to *A. rhizogenes* LBA1334 (Ar1334) in a triparental mating using pRK2013 (kept in HB101 cells) as a helper plasmid<sup>24</sup>. Ar1334 is a derivative of *A. tumefaciens* strain C58C9 with a chromosomal marker for rifampicin resistance and harbours the agropine type pRI855 plasmid equipped with a spectinomycin resistance marker<sup>5</sup>. Ar1334 cells harbouring pAGS *cpsl* and Ar1334 cells carrying pBin19 *psl* were kept on LC plates<sup>24</sup> containing rifampicin (20  $\mu$ g ml<sup>-1</sup>) plus tetracycline (2  $\mu$ g ml<sup>-1</sup>), and rifampicin (20  $\mu$ g ml<sup>-1</sup>) plus kanamycin (100  $\mu$ g ml<sup>-1</sup>), respectively. To check if the binary vectors were present in Ar1334, plasmid DNA was isolated<sup>28</sup> and immediately transformed to MH1-competent cells. Plasmid preparations from *E. coli* and analysis of restriction patterns showed that both binary vectors were maintained in Ar1334 cells. Restriction enzyme digestions, agarose gel electrophoresis, dephosphorylation, annealing of restriction fragments and transformation to *E. coli* followed procedures described elsewhere<sup>29</sup>. Isolation of restriction fragments was performed with glass beads<sup>30</sup> (GeneClean, Bio 101). Restriction sites are denoted: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; EV, *Eco*RV; H, *Hind*III. Other abbreviations: LB, left border; RB, right border; NPTII gene, neomycin-phosphotransferase II gene; P35S, CaMV 35S promoter; Tnos, nopaline synthase terminator.



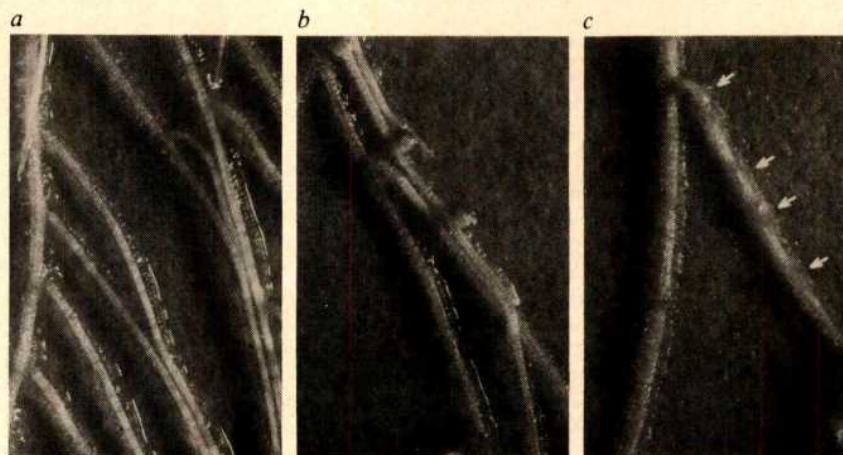
were induced on a set of clover seedlings which were grown on filter paper for 13 weeks in large Petri dishes (15 cm in diameter), filled with 100 ml plant growth medium<sup>14</sup> containing 5 mM KNO<sub>3</sub> and 1% agar (see Table 1). The dot assay for NPTII activity<sup>15</sup> was performed with extracts from randomly chosen hairy roots weighing from 50–100 mg. Hairy roots induced by Ar1334 (or adventitious roots) did not have any NPTII activity, whereas 86% (of a total of 64 samples) of hairy roots induced by Ar1334 harbouring pBin19 *psl* did have NPTII activity. Similar percentages of co-transformation (70–80%) using pBin19 contained in *A. rhizogenes* LBA9402 have been reported for several plant species<sup>16</sup>. The enzymatic assay was also positive for 42% (of a total of 66 samples) of the hairy roots induced by Ar1334 carrying pAGS *cpsl*. These results show that the binary-vector system of transformation is an efficient tool for introducing foreign genes into host plants.

To study the nodulation properties of white clover hairy roots, young hairy roots, 1–3 cm in length, were inoculated with *R. leguminosarum* bv. *trifolii* ANU843 (Rt843) (ref. 17). As with normal young white clover seedlings, nodules emerged 4–6 days

after inoculation on the roots of clover transformed with Ar1334, Ar1334 carrying pBin19 and Ar1334 harbouring pBin19 *psl* or pAGS *cpsl*. Nodules continued to be initiated along the first formed hairy roots and even on hairy roots which emerged 4–6 weeks after transformation. With few exceptions, nodules were red and the acetylene reduction assay<sup>14</sup> indicated that nodules on hairy roots could reduce atmospheric nitrogen. Nodules were distributed on all hairy roots, and plants had 12–100 nodules. Most of the nodules remained small, particularly in heavily nodulated hairy roots. Electron micrographs of these nodules<sup>18</sup> showed no differences with nodules induced by Rt843 on normal white clover roots with respect to the morphology, and the number and shape of bacteroids. These results show that white clover hairy roots behave as normal roots in nodulation, and that nodulation of white clover hairy roots by the homologous symbiont is not in any way inhibited by the presence of the binary vector T-DNA with or without the *psl* gene.

Rifampicin-resistant *R. leguminosarum* bv. *viciae* strain 2<sup>40</sup> (RI248 Rif<sup>R</sup>) (ref. 19) did not nodulate white clover hairy roots induced by Ar1334 or by Ar1334 carrying pBin19 without the

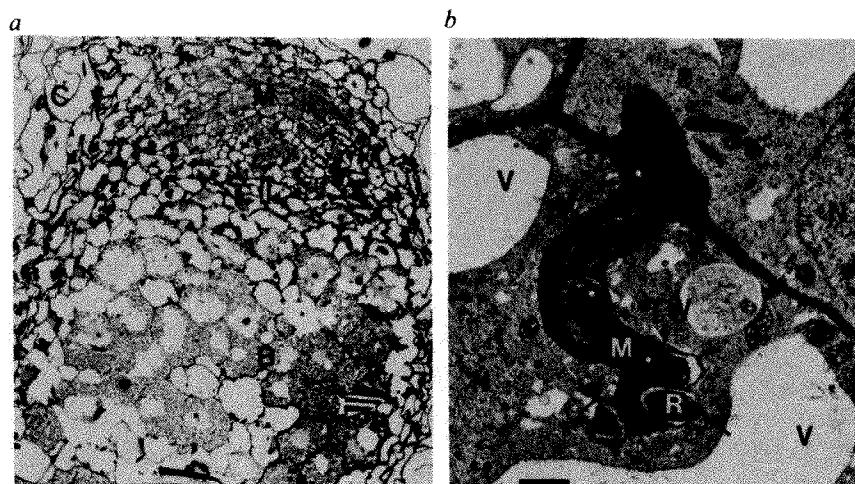
FIG. 2 Nodulation of white clover hairy roots co-transformed with the pea lectin gene by *Rhizobium leguminosarum* bv. *viciae*. Hairy roots induced by Ar1334 or by Ar1334 carrying pBin19 were not nodulated by RI248 Rif<sup>R</sup> (Fig. 2a). The host range of nodulation of white clover hairy roots, co-transformed with the genomic or copy DNA of *psl*, however, is extended to include *R. leguminosarum* bv. *viciae* 248 Rif<sup>R</sup>. Of the nodulated plants co-transformed with pBin19 *psl* or with pAGS *cpsl*, 30% had 1–4 red nodules, whereas 5–10% had 5–12 red nodules. These nodules could not be distinguished from nodules induced by Rt843 on Ar1334-induced hairy roots. Some of the nodules formed after inoculation with RI248 Rif<sup>R</sup> were red as soon as they emerged and matured within 4–8 days (b), others emerged white, grew out in 8–16 days and then turned red. The assay for acetylene reduction<sup>14</sup> was positive, indicating that red nodules induced by RI248 Rif<sup>R</sup> were able to reduce atmospheric nitrogen. All nodulated plants (Table 1) had ~1–50 white, small outgrowths, such as those indicated by arrows in c. In some cases these outgrowths developed



into white or red nodules; in most cases they did not grow further and were categorized as pseudonodules.



FIG. 3 Electron microscopy of red nodules induced by *Rhizobium leguminosarum* bv. *viciae* on white clover hairy roots co-transformed with the pea lectin gene. Adjacent red nodules on co-transformed roots were chosen for rhizobia re-isolation and identification, and processing for electron microscopy<sup>18,23</sup>. Examination of the micrographs showed that the ultrastructure of nodules induced by *Ri248* Rif<sup>R</sup> did not differ from that of nodules induced by *Rt843*. *a*, Longitudinal section of a young nodule formed on a hairy root of a clover plant co-transformed with the complete pea lectin genomic gene. The meristematic (M), infection (I) and bacteroid (B) zones are well distinguished; C is the nodule cortex. Scale bar, 1  $\mu$ m. *b*, A longitudinal section of an infection thread as observed in the infection zone. Note the continuity of the host's plasmalemma (P) delimitating the thread matrix (M) and the gradual decrease of the thread wall material (TW) towards the tip of the infection thread. Rhizobia (R) are seen being released into the host cell cytoplasm by endocytosis (arrow). V, vacuole; N, nucleus. Scale bar, 100  $\mu$ m.



*psl* gene (Fig. 2a, Table 1). Moreover, infection threads could not be observed by microscopical light-examination of methylene blue-stained hairy roots. In both cases, however, white clover hairy roots responded to inoculation with *Ri248* Rif<sup>R</sup> with marked root-hair curling, as has been previously reported for normal clover roots<sup>20</sup>. Forty to sixty days after inoculation the hairy roots of 1 in 20 plants presented one or two white outgrowths. Their examination by electron microscopy, however, failed to reveal the presence of infection threads and they were therefore categorized as pseudonodules. These results show that as with normal roots, nodulation of white clover hairy roots is host-specific. Moreover, the insertion of a functional NPTII gene into co-transformed white clover hairy roots did not affect this specificity.

The *psl* gene appeared to affect the host-range of nodulation. Hairy roots induced with *Ar1334* carrying binary vectors either with the complete genomic *psl* or cDNA construction were inoculated with *Ri248* Rif<sup>R</sup>. Nodules confined to one or two hairy roots and their lateral roots (Fig. 2b) began to emerge 20 days after inoculation (Table 1). Such a distribution of nodules is accounted for by (1) not all the roots of a transformed seedling being hairy roots, and (2) not all hairy roots containing the pea lectin gene. These two types correspond to the roots of non-transformed clover plants and to hairy roots induced by *Ar1334* by *Ar1334* containing pBin19, respectively, which are both resistant to infection by *Ri248* Rif<sup>R</sup>. Electron micrographs of red nodules (Fig. 3) showed that their structures were similar to those of nodules induced by *Ri248* Rif<sup>R</sup> on *Vicia sativa* roots and by *Rt843* on *Trifolium repens* roots. The presence of stunted nodules, or pseudonodules (Fig. 2c) however, indicated that in many cases infection was aborted. Electron micrographs of those pseudonodules revealed degenerated nodule meristems, bacteria in intercellular spaces deep in the root cortex and aborted infection threads.

Rhizobia were re-isolated from red nodules on hairy roots that had been co-transformed with the *psl* gene (Table 1). The heterologous symbiont *Ri248* Rif<sup>R</sup> was identified using five criteria: (1) the rate of growth on B-agar plates, (2) antibiotic resistance; (3) positive reaction with a monoclonal antibody specific for the O-antigen of *Ri248* (ref. 21); (4) SDS-PAGE pattern of cell envelope proteins; and (5) nodulation of *V. sativa* L. and failure to nodulate *T. repens* L<sup>5</sup>. From this identification we concluded that *Ri248* Rif<sup>R</sup> is able to induce root nodules on white clover hairy roots co-transformed with the *psl* gene. Nodulation, however, is delayed and most nodules are abnormal.

Our results show that the host range in *Rhizobium*-legume symbiosis is at least partially determined by symbiont-root-

lectin interactions. The introduction of the gene encoding the glucose-/mannose-binding pea lectin in clover roots allows infection by *R. leguminosarum* bv. *viciae* to proceed beyond root-hair curling. This indicates that root lectin contributes to a mechanism by which the plant selectively allows a homologous *Rhizobium* to penetrate root cells and induce infection threads. Our results also provide the first example of a host-specificity barrier in a plant-bacterium interaction being broken by genetic engineering of the host plant. Interaction of host lectin with the homologous symbiont might either induce increased infectivity of the rhizobia<sup>22</sup> or enable collective endocytosis of rhizobia in the root hair curl<sup>23</sup>. It is also conceivable that the introduction of *psl* to clover induces a response to *R. leguminosarum* bv. *viciae* bacteria which does not directly involve pea lectin. The fact that lectins with a sugar specificity similar to pea lectin are specifically present in plants of the pea cross-inoculation group, however, suggests that this is unlikely. □

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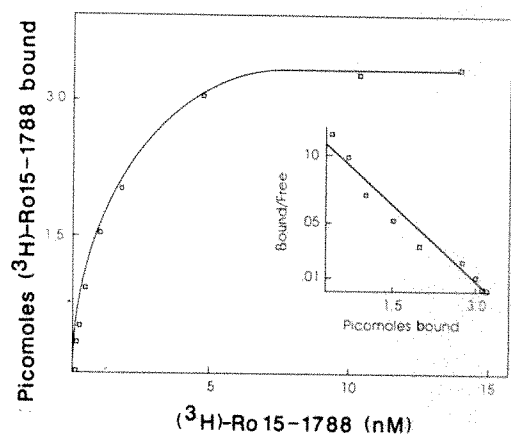


FIG. 2 Saturation isotherm of [ $^3\text{H}$ ]Ro15-1788 binding. Inset, the corresponding Scatchard plot ( $K_d = 0.97 \text{ nM}$ ,  $B_{\text{max}} = 3.2 \text{ pmol mg}^{-1} \text{ protein}$ ). In three independent transfection experiments  $K_d$  values of  $1 \pm 0.2 \text{ nM}$  and  $B_{\text{max}}$  values of  $2.7 \pm 0.7 \text{ pmol mg}^{-1} \text{ protein}$  were obtained.

**METHODS.** Human  $\alpha 1$ -,  $\beta 1$ - (ref. 11) and  $\gamma 2$ -encoding cDNAs, subcloned individually into the expression vector pCIS2 (ref. 27), were used to transform human embryonic kidney 293 cells as described previously<sup>5,28</sup>. After transfection (48 h), cells from ten plates (10 cm;  $4 \times 10^6$  cells per plate) were washed twice with phosphate-buffered saline (PBS) and scraped into PBS (10 ml). The cell pellet (500g) was homogenized in a Polytron tissue homogenizer (Brinkmann) in 10 ml of 10 mM potassium phosphate, pH 7.4, and centrifuged (50,000g, 20 min). This procedure was repeated three times and the final pellet resuspended in potassium phosphate buffer, pH 7.4, containing 100 mM KCl. For each ligand concentration, triplicate homogenates, each equivalent to  $10^6$  cells (100  $\mu\text{g}$  protein), were incubated (4  $^{\circ}\text{C}$ , 60 min) in a 1 ml reaction volume with [ $^3\text{H}$ ]Ro15-1788 (NEN, 75 Ci mmol $^{-1}$ ). Non-specific binding was determined in the presence of  $10^{-6} \text{ M}$  clonazepam and was  $<10\%$  of total binding.  $K_i$  values of clonazepam, diazepam, and DMCM (see text) were determined in similar reactions using  $10^{-10}$ – $10^{-5} \text{ M}$  unlabelled competitor compounds. Samples were vacuum-filtered on GF/C filters with a vacuum filter apparatus (Biorad) and filters were washed twice with 5 ml homogenization buffer. After drying, filter-retained radioactivity was determined by liquid scintillation counting.

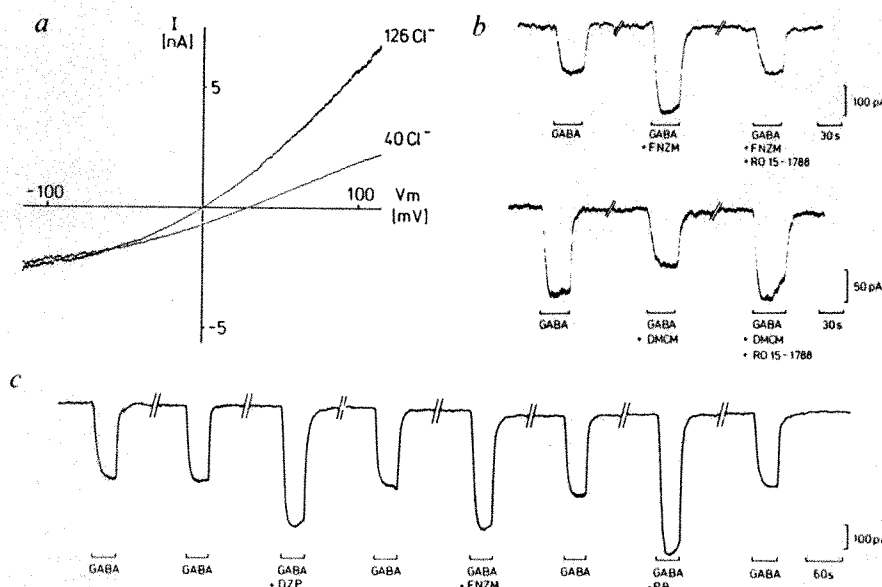
domain, as well as four transmembrane segments. The greatest sequence similarity to  $\alpha$ - and  $\beta$ -subunits occurs in the region of these transmembrane segments and includes the charged amino-acid residues thought to form the channel mouth<sup>3,7</sup>. The proposed large intracellular domain displays the highest sequence divergence among the subunits. In the  $\gamma 2$  subunit, this domain contains a consensus sequence for tyrosine-specific protein phosphorylation, suggesting a target for the cellular control of channel activity by receptor-mediated tyrosine kinases<sup>8</sup>. A putative control site for serine phosphorylation by protein kinase A is found within the same domain of the  $\beta 1$  subunit<sup>3</sup>. The overall sequence similarity to  $\alpha 1$  and  $\beta 1$  subunits is 42 and 35%, respectively. This level of structural similarity is seen for the different nicotinic acetylcholine receptor subunits<sup>9</sup> and is significantly below the 70–80% identity which typically characterizes the variants of GABA $_A$  receptor  $\alpha$ -subunits<sup>3,4</sup> or  $\beta$ -subunits (manuscript in preparation). Based on these structural considerations and the functional signature detailed below, the

$\gamma 2$  polypeptide is a novel GABA/benzodiazepine receptor subunit.

To determine the functional properties of the  $\gamma 2$  subunit, the cloned cDNA was transiently expressed in cultured human cells<sup>5</sup>, either singly or together with cloned cDNA encoding human  $\alpha 1$  and  $\beta 1$  subunits<sup>11</sup>. The membranes of the transfected cells were analysed for the binding of radiolabelled ligands specific for central benzodiazepine receptors<sup>2,10</sup>. High-affinity binding sites for [ $^3\text{H}$ ]Ro15-1788 ( $K_d = 1.2 \pm 0.2 \text{ nM}$ ,  $B_{\text{max}} = 2,900 \pm 300 \text{ fmol mg}^{-1}$ ; Fig. 2) and [ $^3\text{H}$ ]flunitrazepam ( $K_d \sim 2 \text{ nM}$ ,  $B_{\text{max}} \sim 2,000 \text{ fmol mg}^{-1}$ ; data not shown) were observed on cells expressing all three GABA $_A$  receptor subunits. The binding of these labelled compounds was completely blocked in the presence of  $1 \mu\text{M}$  diazepam ( $K_i = 10 \pm 2 \text{ nM}$ ), clonazepam ( $K_i = 1.1 \pm 0.7 \text{ nM}$ ) or methyl-4-ethyl-6,7-dimethoxy- $\beta$ -carboline-3-carboxylate (DMCM;  $K_i = 2.1 \pm 0.8 \text{ nM}$ ; data not shown). The  $K_i$  values of these compounds are similar to their rank order and potency determined on cerebellar membranes<sup>12</sup>. No

FIG. 3. Ionic and pharmacological characteristics of GABA-induced membrane currents in 293 cells expressing human GABA $_A$  receptor  $\alpha 1$ ,  $\beta 1$  and  $\gamma 2$  subunits. **a**, Current-voltage relation of GABA-induced responses (GABA,  $100 \mu\text{M}$ ). A reversal potential close to 0 mV was seen in  $126 \text{ mM} [\text{Cl}^-]_o$ . When  $[\text{Cl}^-]_o$  was lowered to  $40 \text{ mM}$  the reversal potential shifted to 30 mV, indicating a highly chloride-selective conductance. **b**, Flunitrazepam (FNZM,  $1 \mu\text{M}$ ) reversibly potentiated the GABA-induced current (GABA,  $5 \mu\text{M}$ ). This potentiation was completely inhibited by Ro15-1788 ( $1 \mu\text{M}$ ). DMCM ( $1 \mu\text{M}$ ) reduced the GABA-induced current by  $\sim 50\%$ . This reduction was lost in the presence of Ro15-1788 ( $1 \mu\text{M}$ ). **c**, Diazepam (DZP,  $1 \mu\text{M}$ ), FNZM ( $1 \mu\text{M}$ ) and pentobarbital (PB,  $50 \mu\text{M}$ ) all reversibly potentiated the current induced by  $10 \mu\text{M}$  GABA (// indicates a 5-min interval between subsequent applications).

**METHODS.** Membrane currents were recorded by the patch-clamp technique in the whole cell configuration<sup>13</sup>, using an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt). Cells were clamped at  $-60 \text{ mV}$ . Data were filtered at 3 kHz (8-pole Bessel) and sampled up to 10 kHz. To determine the reversal potential of the transmitter-induced responses, membrane currents were measured while applying a voltage ramp ( $-120$  to  $120 \text{ mV}$ , 1.6 s duration). The current-voltage curves were calculated by subtracting currents ( $I$ ) before application of GABA from currents obtained in the presence of GABA, and were plotted as a function of membrane potential ( $V_m$ ). Substances were added, as indicated by bars, at the following concentrations: FNZM, DZP, Ro15-1788, and DMCM,



$1 \mu\text{M}$ ; PB,  $50 \mu\text{M}$ . Pipettes contained in mM: CsCl, 130; MgCl $_2$ , 1; CaCl $_2$ , 0.5; EGTA, 5; HEPES, 10; pH, 7.2. Cultures were continuously perfused with a solution containing in mM: KCl, 5.4; NaCl, 116; MgCl $_2$ , 0.8; CaCl $_2$ , 1.8; D-glucose, 11.1; NaHCO $_3$ , 26.2; HEPES, 5; pH, 7.2. All measurements were performed at room temperature.



binding was detected when the  $\gamma 2$  subunit alone or pairs of subunits were expressed. These data show that  $\alpha$ -,  $\beta$ - and  $\gamma$ -subunits can assemble into GABA<sub>A</sub> receptors which contain a high-affinity benzodiazepine binding site.

The receptors formed by co-expression of three different subunits were characterized by electrophysiology, using the whole cell patch-clamp technique<sup>13</sup> (Fig. 3). The application of GABA induced large inward currents; GABA sensitivity showed a Hill coefficient of  $\sim 1$ , as observed upon co-expression of  $\alpha$ - and  $\beta$ -subunits<sup>4</sup>. The selectivity for chloride ions is demonstrated by a shift in the current versus voltage curve when changing the concentration of extracellular chloride. The GABA-induced current was blocked by the GABA<sub>A</sub> receptor antagonist bicuculline ( $10^{-5}$  M) and the channel blocker picrotoxin ( $10^{-4}$  M) (data not shown) but was potentiated by the barbiturate pentobarbital. Importantly, these receptors display the full functional properties of GABA/benzodiazepine receptors. In all expressing cells tested, responses to GABA were consistently enhanced about

twofold by the benzodiazepine receptor agonists flunitrazepam and diazepam and reduced by 50% in the presence of the inverse agonist DMCM<sup>2,14</sup>. These effects were completely blocked by the benzodiazepine receptor antagonist Ro15-1788. This spectrum of sensitivity was not observed upon co-expressing  $\alpha 1\gamma 2$  and  $\beta 1\gamma 2$  subunit pairs. Furthermore, no responsiveness was seen on expression of the  $\gamma 2$  subunit alone which generated homomeric receptors (data not shown) comparable in their properties to the receptors formed from single  $\alpha$ - or  $\beta$ -subunits<sup>5</sup>. Our results indicate that all three subunits contribute to the formation of GABA/benzodiazepine receptors.

The  $\gamma 2$  subunit is comparable in abundance to  $\alpha$ - and  $\beta$ -subunits, as judged from the number of cognate cDNA clones in libraries of rat, bovine and human CNS origin and as evidenced by northern analysis of rat and bovine brain mRNA (unpublished observations). To determine the spatial pattern of  $\gamma 2$  mRNA expression in the CNS, we performed *in situ* hybridization on sagittal sections of rat brain, using as a probe a radioac-

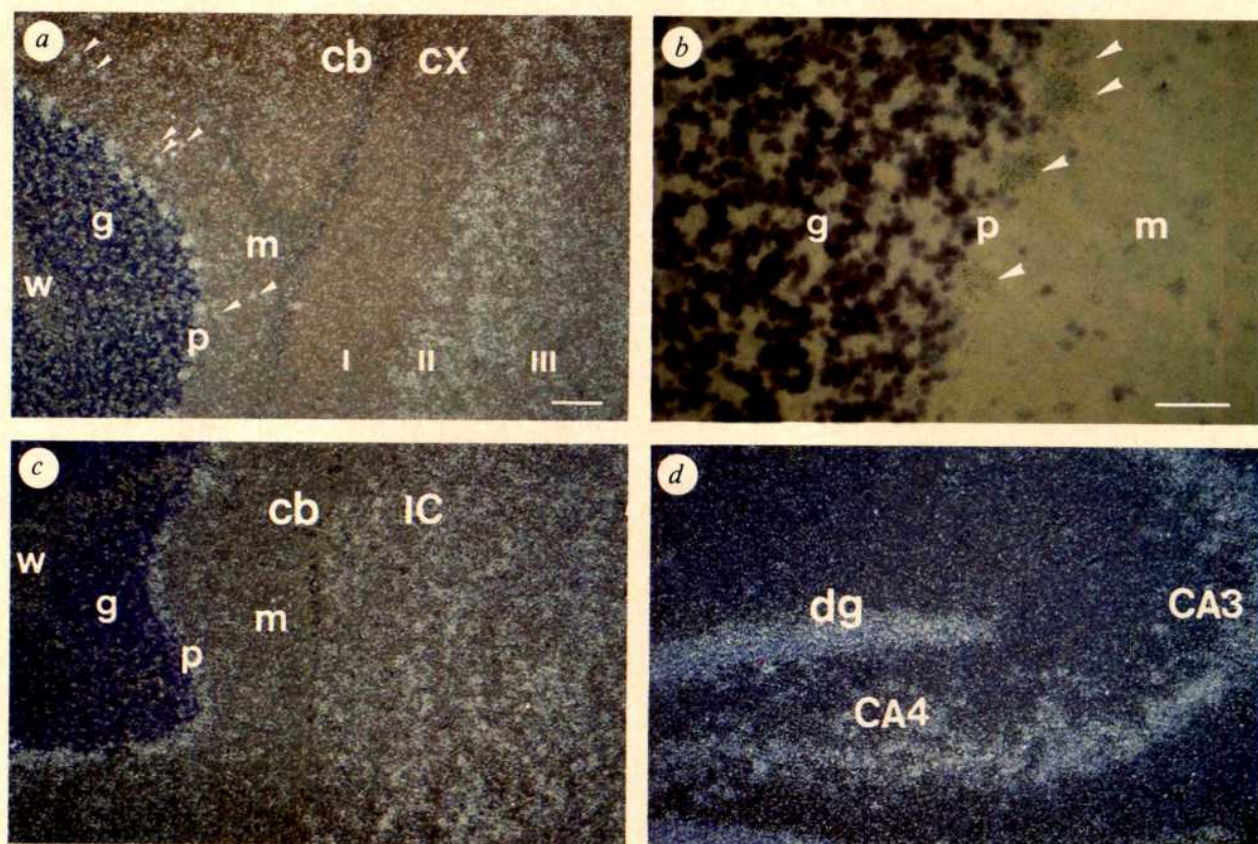


FIG. 4 Location of neurons synthesizing  $\gamma 2$  subunit mRNA in rat brain. *In situ* hybridization was performed using a  $^{35}\text{S}$ -labelled cRNA on frozen, paraformaldehyde-fixed brain sections. Grain clusters in the emulsion mark the location of cells containing hybridizing mRNA in stained sections. Sections probed with  $^{35}\text{S}$ -labelled sense RNA produced no hybridization pattern (data not shown). *a*, Darkfield photomicrograph of cerebellar cortex (cb, left) and neocortex (cx, right). In cerebellar cortex,  $\gamma 2$  subunit mRNA expression is highest in the Purkinje neurons in the Purkinje cell layer (p) with an additional population of hybridizing cells (arrowheads) in the molecular layer (m) representing presumptive basket/stellate neurons. In the neocortex, numerous cells throughout layers II–VI express the  $\gamma 2$  subunit mRNA highly and display no obvious lamination pattern; neocortical layers I–III are shown. *b*, Bright-field photomicrograph of  $\gamma 2$  subunit mRNA expression in all Purkinje neurons (arrowheads). *c*, Dark-field photomicrograph of cerebellar cortex (left) and of the inferior colliculus (ic, right). Numerous cells expressing the  $\gamma 2$ -subunit mRNA are distributed throughout the inferior colliculus, unlike the superior colliculus (not shown). *d*, Dark-field photomicrograph of part of the hippocampal formation showing high  $\gamma 2$  subunit mRNA expression in granule neurons of the dentate gyrus (dg) and pyramidal neurons of the hippocampal CA3 and CA4 regions. CA1 and CA2 pyramidal neurons (not shown) similarly express  $\gamma 2$ -subunit mRNA. Scale bar in *a* (for *a*, *c* and *d*),

100  $\mu\text{m}$ ; in *b*, 30  $\mu\text{m}$ . Other abbreviations: *g*, granule cell layer; *w*, white matter.

**METHODS.** RNA probes were transcribed from a linearized Bluescript plasmid DNA, containing a 1,500-base pair *EcoRI* fragment from the coding region of rat  $\gamma 2$  cDNA. Following plasmid linearization at unique restriction sites in the polylinker region, sense or antisense RNA was synthesized using phage T3 or T7 RNA polymerase. RNA products were radiolabelled<sup>29</sup> to a specific activity of  $\sim 3 \times 10^8$  c.p.m.  $\mu\text{g}^{-1}$  using 1.0  $\mu\text{g}$  linearized plasmid DNA, 50  $\mu\text{Ci}$   $\alpha[^{35}\text{S}]\text{CTP}$  ( $1,000 \text{ Ci mmol}^{-1}$ , Amersham), 2.5 mM each of ATP, GTP and UTP, and were hydrolysed (pH 10, 37  $^{\circ}\text{C}$ , 1 h) to an average length of 100 nucleotides. For each section the RNA probe ( $2.5 \times 10^5$  c.p.m.) was dissolved in 50  $\mu\text{l}$  hybridization solution which included 0.6 M NaCl, 50% formamide, and 40 mM  $\beta$ -mercaptoethanol. *In situ* hybridization was performed as described previously<sup>30</sup>. Briefly, the sections were hybridized at 42  $^{\circ}\text{C}$  for 3 days, washed twice for 20 min each at 65  $^{\circ}\text{C}$  in  $0.1 \times \text{SSC}$ , 0.05% inorganic pyrophosphate, 14 mM  $\beta$ -mercaptoethanol, and dehydrated in alcohol. Following overnight exposure to Kodak XAR 5 film, the sections were dipped in Kodak NTB2 emulsion (diluted 1:1 in water), exposed for 8 days, and stained in 1% Fast Green and 0.5% cresyl violet. Sagittal brain structures were identified from ref. 31.



tively labelled cRNA derived from cloned rat  $\gamma 2$  cDNA (manuscript in preparation). Our results show that  $\gamma 2$  mRNA is prominently expressed in neuronal subsets throughout the CNS which include neurons in the olfactory bulb, anterior olfactory nuclei, preoptic area, neocortex, globus pallidus, hippocampus, dentate gyrus, thalamus, inferior colliculus, substantia nigra, pontine nuclei, cerebellar cortex and cerebellar nuclei. Four of these regions have been chosen to illustrate the cellular location of  $\gamma 2$  mRNA (Fig. 4). Significantly, all of these regions contain high-affinity binding sites for benzodiazepines<sup>15,16</sup> and also express  $\alpha$ - and  $\beta$ -subunit mRNAs (refs 17, 18; B.D.S., unpublished observations), further supporting the hypothesis that the  $\gamma 2$  subunit is an integral part of GABA/benzodiazepine receptors.

The existence of the  $\gamma 2$  subunit was not anticipated from biochemical studies as subunits of affinity-purified GABA/benzodiazepine receptor are electrophoretically resolved into only two main bands corresponding to relative molecular mass ( $M_r$ ) 48,000–53,000 (48K–53K) ( $\alpha$ ) and  $M_r$  55–57K ( $\beta$ ) (ref. 1). These bands, however, are heterogeneous, consisting of variants of the  $\alpha$ -subunits<sup>3,4</sup> and  $\beta$ -subunits, and of additional GABA<sub>A</sub> receptor subunits, including  $\gamma 2$  and a related  $\gamma 1$  subunit, whose cloning preceded that of  $\gamma 2$  and which shows glial localization (manuscript in preparation). The mature  $\gamma 2$ -polypeptide (unglycosylated,  $M_r \approx 48$ K) may co-migrate with  $\alpha$ -subunits ( $M_r$ , 48–53K)<sup>1,4</sup>, which have been postulated to carry the benzodiazepine site on the basis of photoaffinity labelling with flunitrazepam<sup>21–23</sup>. Our results on benzodiazepine responsiveness indicate that the  $\gamma$ -subunit contributes to the formation of the benzodiazepine site and thus may also be photoaffinity-labelled. In fact, it is probable that a flunitrazepam-labelled  $\gamma 2$  subunit would be indistinguishable from certain labelled  $\alpha$ -subunits by present methods.

We note that other subunit combinations may also create benzodiazepine responsiveness. Indeed, recent cDNA cloning experiments in our laboratory provide evidence for the existence of additional GABA<sub>A</sub> receptor subunits (manuscript in preparation). It seems likely therefore that the true diversity of GABA/benzodiazepine receptor subtypes has been only partly revealed by classical pharmacology<sup>12,19,20</sup>. □

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## Vaccination against ovine cysticercosis using a defined recombinant antigen

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CYSTICERCOSIS caused by larval tapeworms is a major public health problem and a cause of substantial economic losses in the farm-animal industries. *Taenia ovis* in sheep is a particularly important example. Immunity to reinfection with the larvae has a central role in regulating natural transmission of the parasites<sup>1</sup>, and vaccination with antigens from the early larval oncosphere stage can induce complete protection against infection<sup>2</sup>. As it is impractical to obtain enough oncospheres for a commercial vaccine against these tapeworms, an alternative approach is to use recombinant DNA methods to generate a cheap and plentiful supply of antigens. We report here the expression in *Escherichia coli* of complementary DNA encoding *T. ovis* antigens as fusion proteins with the *Schistosoma japonicum* glutathione S-transferase. Vaccination of sheep with these fusion proteins gave significant, although not complete, immunity against challenge infection with *T. ovis* eggs. Commercial development of a vaccine is being pursued.

Oncospheres of *T. ovis* were chosen as the source of messenger RNA for constructing a cDNA library because they are known to be a rich source of host-protective antigens<sup>3,4</sup>. Furthermore, *in vitro* culture techniques<sup>5</sup> have shown that hatched and activated *T. ovis* oncospheres secrete potent host-protective antigens<sup>6,7</sup>, suggesting synthesis is active. Oncospheres were therefore treated with artificial gastric and intestinal fluid and incubated before extraction of mRNA. To reduce the number of antigen-expressing cDNA clones to be tested in vaccination trials in sheep, it was first necessary to identify probable candidates for host-protective antigens. Preliminary experiments with sera from immune sheep showed strong antibody recognition of oncosphere antigens of relative molecular mass ( $M_r$ ) 47,000–52,000 (47–52K) (unpublished observations). The host-protective nature of the 47–52K antigens was demonstrated directly when lambs immunized with an SDS-PAGE cut-out of this region were protected significantly (98%) against challenge infection (Fig. 1). To isolate the genes encoding these antigens, rabbit antibodies specific for the 47–52K region were eluted from western blots and used to screen the expression library<sup>8</sup>. Figure 1 shows that the antibody probe was specific for the 47–52K region, and enabled us to isolate two clone types, designated 45S and 45W. Antigen- $\beta$ -galactosidase ( $\beta$ -gal) fusion proteins prepared from the clones ( $\beta$ -gal-45S and  $\beta$ -gal-45W) failed to stimulate host-protective immunity in sheep even though antibody was produced to the 47–52K antigens (Fig. 1). This experiment, however, demonstrated that the 47–52K region contains a series of serologically-related molecules, and that the antigens, or parts of them, had been cloned.

Plasmid vectors have been constructed recently which express antigens as fusion proteins with the enzyme glutathione S-transferase (GST) of *Schistosoma japonicum*, thereby enabling

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affinity-purification under non-denaturing conditions<sup>9</sup>. Fusion proteins GST-45S and GST-45W were prepared using a prototype of the vectors, pSj10Δ*Bam*7Stop7, and tested either separately or together in an immunization trial in lambs (Table 1, trial A). The results showed that GST-45W, either alone or in combination with GST-45S, stimulated significant host-protective immunity compared with the GST controls, whereas GST-45S alone was ineffective. The active component was therefore GST-45W, and a further vaccination trial was carried out using different adjuvants and doses of GST-45W. Significant levels of immunity were induced at all dose levels in oil, Freund's complete adjuvant or saponin adjuvants, the best being 94% protection with 50 μg GST-45W in saponin (Table 1, trial B).

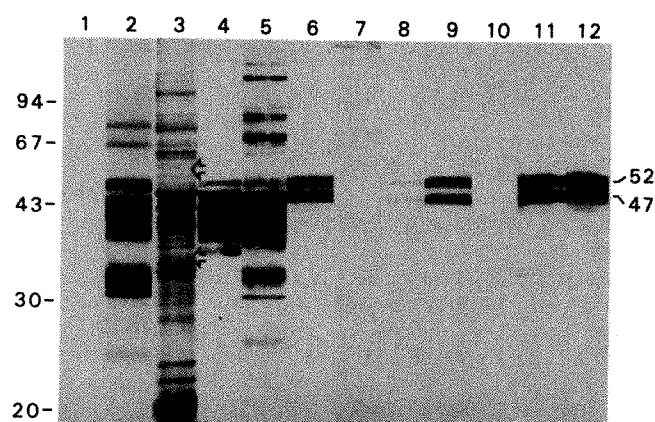


FIG. 1 Immunoblotting analysis of *T. ovis* oncosphere antigens. Lanes probed with: pooled sera from five sheep before immunization (1); pooled sera from five sheep after immunization with an extract of 200,000 oncospheres solubilized in 2% SDS and 65 mM dithiothreitol (2); pooled sera from three sheep immunized with gel cut-out fraction (4); serum from a rabbit hyperimmunized with oncosphere extract (5); affinity-purified antibodies eluted from blots containing antigens with relative mobilities of 47–52K (6); pooled sera from three sheep immunized with  $\beta$ -gal (7); pooled sera from three sheep immunized with  $\beta$ -gal-45S (8); pooled sera from three sheep immunized with  $\beta$ -gal-45W (9); pooled sera from four sheep immunized with GST (10); pooled sera from four sheep immunized with GST-45S (11); pooled sera from four sheep immunized with GST-45W (12). Lane 3 shows oncosphere extract stained with silver<sup>16</sup> and the approximate size of gel cut-out injected into sheep is indicated between the arrows. Mean numbers of *T. ovis* cysticerci found in sheep injected with gel cut-out fraction, control polyacrylamide gel or whole oncosphere antigen in SDS plus gel were 1.0, 55.0 and 8.0, respectively. The sheep injected with the oncosphere gel cut-out were significantly ( $P < 0.01$ , 't' test) protected against infection.

**METHODS.** For vaccination, an extract from  $7 \times 10^6$  oncospheres was separated by SDS-PAGE through a 5–25% polyacrylamide gradient<sup>17</sup> and the fraction cut as shown. The fraction was homogenized with oil adjuvant<sup>15</sup> and injected part subcutaneously and part intramuscularly into three sheep on two occasions, 2 weeks apart. Positive control sheep were injected with homogenized polyacrylamide gel containing whole oncosphere antigen (three sheep) and negative control sheep were immunized with homogenized polyacrylamide gel without antigens (four sheep). Two weeks after the second injection sheep were infected with 5,000 viable *T. ovis* eggs and 6 weeks later carcasses were examined for cysts. For analysis, oncosphere antigens were separated by SDS-PAGE through 12–18% polyacrylamide gradients. Electrophoretic transfer to nitrocellulose<sup>18</sup> was followed by blocking for 2 h in 2% casein hydrolysate (Oxoid) 0.5% Tween 20 in 20 mM Tris-HCl buffer pH 7.4, containing 0.5 M NaCl (TBS). Sera were diluted 100-fold in 1% fish gelatin (Norland Products, USA) in TBS and incubated with blocked antigen strips overnight on a rocking platform. Strips were washed four times with TBS containing 0.05% Tween 20, and bound antibodies detected by incubation for 4 h with peroxidase-conjugated rabbit anti-sheep IgG (Cappel, Cooper Biomedical Inc.) or goat anti-rabbit IgG (Cappel) followed by washing and visualization with 4-chloro-1-naphthol (Biorad). Affinity-purified antibodies to the 47–52K antigens were prepared by elution of rabbit antibody from blots using 0.1 M glycine-HCl, pH 2.5 (ref. 8). Eluted antibodies were neutralized and fish gelatin carrier protein was added to 1% before concentration. The relative mobilities of standards are shown in the margins.

Although control sheep immunized with GST in saponin were not included in this trial, several experiments have since been carried out using saponin plus GST controls, with no non-specific protective effect being observed (unpublished observations).

Treatment of the fusion proteins with SDS and dithiothreitol substantially reduced protection (Table 1) indicating that the conformation of the fusion protein is important to its activity. Western blots of oncosphere antigen probed with sera from vaccinated sheep showed that antibody was directed predominantly against the 47–52K region (Fig. 1). The complete nucleotide sequence of the 928-base pair 45W cDNA is shown in Fig. 2. There is a single open reading frame in the sequence,

TABLE 1 Vaccination of sheep with glutathione S-transferase fusion proteins

Group	No. cysticerci in sheep	Mean	% Protection
<b>Trial A</b>			
Oncosphere antigen	0, 1, 1, 1	0.75	99
Buffer control	68, 95, 105, 109	94	
GST-45W + GST-45S	0, 15, 18, 59	23*	73
GST-45W + GST-45S	31, 36, 56, 141	66	22
SDS treated			
GST-45S	40, 74, 104, 161	95	—
GST-45W	11, 12, 24, 68	29*	66
GST	46, 69, 106, 116	84	
<b>Trial B</b>			
Oncosphere antigen	0, 0, 0, 0	0	100
GST oil	11, 16, 17, 18, 20		
	25, 29, 45, 48, 57	29	
GST-45W 50 μg	0, 0, 0, 0, 2		
oil	4, 6, 16, 18, 25	7†	75
GST-45W 300 μg	0, 0, 1, 7, 9	3*	88
oil			
GST-45W 50 μg	0, 0, 0, 1, 7	2†	94
saponin			
GST-45W 50 μg	0, 0, 0, 2, 29	6*	79
FCA			
GST FCA	14, 15, 28, 44, 46	29	

*T. ovis* eggs recovered from freshly purged tapeworms from dogs were washed thoroughly and stored at 4 °C for no more than 1 week. After hatching and activation<sup>15</sup>, the oncospheres were maintained *in vitro* for 2–3 h in DMEM, washed twice in sterile physiological saline, frozen and stored in liquid nitrogen. Approximately  $30 \times 10^5$  oncospheres were ground to a paste in liquid nitrogen and RNA isolated after solubilization in guanidine-HCl (ref. 10). Pooled total RNA (20–50 μg) was fractionated over oligo(dT) cellulose<sup>11</sup>. Polyadenylated RNA (400 ng) was transcribed into double-stranded cDNA using AMV reverse transcriptase and ribonuclease RNase H (Amersham cDNA synthesis system RPN.1256), methylated with *Eco*RI methylase (New England Biolabs) and ligated to *Eco*RI linkers (5'-GGAATTCC-3') (New England Biolabs). The cDNA was cloned<sup>12</sup> into  $\lambda$ gt11 yielding a library of  $7.5 \times 10^5$  individual recombinants. Fifty thousand phage plaques were screened<sup>12</sup> with affinity-purified anti-47–52K antibodies (Fig. 1) and five clones were identified, three of which were strongly immunoreactive and demonstrated to be siblings by direct sequencing. This clone type was designated  $\lambda$ gt11 45S. The other two clones were weakly immunoreactive and similarly grouped into a single class called  $\lambda$ gt11-45W. The inserts from the 45S and 45W  $\lambda$ gt11 clones were ligated into *Eco*RI-cleaved pSj10Δ*Bam*7Stop7 (ref. 9) and transformed into *E. coli* strain JM109 (ref. 14). Fusion proteins were prepared from these constructs, after transformation, by affinity chromatography on glutathione agarose<sup>9</sup> (Sigma) and stored at –70 °C before use in the vaccination trials. **Trial A.** The fusion proteins, oncosphere antigen, and GST control protein were emulsified in oil adjuvant<sup>15</sup> and injected subcutaneously into groups of four sheep on three occasions, 3 weeks apart. Total protein in the three doses of fusion protein amounted to 50 μg per sheep based on  $A_{280}$  estimation<sup>9</sup>. Solubilized oncosphere antigen equivalent to 150,000 oncospheres was injected in oil adjuvant in each positive control sheep. Three weeks after the third injection, all sheep were infected with ~5,000 viable *T. ovis* eggs and 6 weeks after infection, sheep carcasses were examined for cysts. **Trial B.** In the second trial sheep were immunized with three injections of GST-45W in oil adjuvant or with 1 mg Saponin HP2 (PPF) injected subcutaneously; or with Freund's complete adjuvant (FCA, Difco) intramuscularly on first injection, and with the second and third injections subcutaneously in incomplete adjuvant. Control positive sheep were immunized with oncosphere antigen as in Trial A. Two weeks after the third injection all sheep were infected with ~2,000 viable *T. ovis* eggs and 6 weeks after infection sheep carcasses were examined for cysts. The challenge infection with *T. ovis* eggs was deliberately decreased compared with trial A to mimic more closely a field infection. Despite the difference in challenge dose a similar per cent protection was observed in the GST-45W (oil adjuvant) groups of trials A and B.

— No protection relative to GST controls.

\* Significantly different from buffer and GST controls at  $P < 0.05$  ('t' test).

† Significantly different from buffer and GST controls at  $P < 0.01$  ('t' test).

EcoRI	
1	GAATTC CCG GAC TAC GAA CAA CCC ATC GAG AGA ACA GTG GTA GAA Pro Asp Tyr Glu Gln Pro Ile Glu Arg Thr Val Val Glu 13
5	TAT CCA TCA CTA CGT GAC ATC TTT GCT TGG GAA CCT CCG ACT TCT Tyr Pro Ser Leu Arg Asp Ile Phe Ala Trp Glu Pro Pro Thr Ser 28
91	AAC TCC ATT GGC CTA ACT TGG CAA AGG CAT GCA TTT CCT GGT GTG Asn Ser Ile Gly Leu Thr Trp Gln Arg His Ala Phe Pro Gly Val 43
136	GAA CGT GAA GTG CTC ACA TTG AAG GCA GTG CCG ACT TCT GAA CCC Glu Arg Glu Val Leu Thr Leu Lys Ala Val Pro Thr Ser Glu Pro 58
181	AAT AAC ACC AAG ACA GCA TAT GCA AAG CTC GGC AGC GGA AAA GTC Asn Asn Thr Lys Thr Ala Tyr Ala Lys Leu Gly Ser Gly Lys Val 73
226	ACT CTT GAT GGA CTG AAG CCC AAT GCC ACA TAT CTT GTG ACT GCG Thr Leu Asp Gly Leu Lys Pro Asn Ala Thr Tyr Leu Val Thr Ala 88
271	ACG GCA AAT ATA AGT GGA GAC ACA ATT CTG GTA TTG AGC AAT ACT Thr Ala Asn Ile Ser Gly Asp Thr Ile Leu Val Leu Ser Asn Thr 103
316	TTT CAT ACA CTG GCC AAT GGC ACA AAT ATT ATA AAT AAC ATC TTC Phe His Thr Leu Ala Asn Gly Thr Ser Ala Thr Ile Asn Asn Ile Phe 118
361	CAT TGG GGT CCT GTG ACT AAT CAA TCA ATT CAA GTA AGA TGG GAT His Trp Gly Pro Val Thr Asn Gln Ser Ile Gln Val Arg Trp Asp 133
406	CAG ATA AAA CCG GAG GAA ACA AGC GCT CTG ATA GTC ACA CTG ACG Gln Ile Lys Pro Glu Glu Thr Ser Ala Leu Ile Val Thr Leu Thr 148
451	GCA GAG ATG GCT TCT GAC CCC GGA GTG GAA AGA TCG GAG TCT GCA Ala Glu Met Ala Ser Asp Pro Gly Val Glu Arg Ser Glu Ser Ala 163
496	CTC TTC GGT AAA GGA AAG GTC ACT GTT GAC GGA CTG GAG TCC GAC Leu Phe Gly Lys Gly Lys Val Thr Val Asn Gly Leu Glu Ser Asp 178
541	ACA CTA TAT ATT GCG ACT GTG ATG GTA TTT AGA AAT GGA AGG CAA Thr Leu Tyr Ile Ala Thr Val Met Val Phe Arg Asn Gly Arg Gln 193
586	TAC TTC AAT TCC ACC AGA GAT ATT CGA ACA CTC AAA TCT GGC CAT Tyr Phe Asn Ser Thr Arg Asp Ile Arg Thr Leu Lys Ser Gly His 208
631	AAG GAG GTA ACA GTC GTA ACA ACT AGT GGA TCT GGT ATA GCC TCC Lys Glu Val Thr Val Thr Thr Ser Gly Ser Gly Ile Ala Ser 223
676	ACA ATA CTT GGA CTC CTC ACC TGC GCG CTA GTC CTT GCT Thr Ile Leu Gly Leu Leu Leu Thr Cys Val Ala Leu Val Leu Ala 238
721	TGAACACTTGGCTGGTCAATGCCCATTTCCAAACCATCCATCTTCAATCTCACG ---
779	TCCCATGACTTGCTGTCTGACACCACTCTTTCTACCTTGACGCACTCATGGTGTGCG
839	GAGTGGCCCTCTCCCTACTCATTCTTGCTCACTAATATTGGCTTGACACCTCTTGATG
898	GATAACCACTGAATGGCAAAATAACGAATTC EcoRI

FIG. 2 Nucleotide sequence and predicted amino-acid sequence of the 45W *EcoRI* cDNA. The DNA sequence of 928 nucleotides is numbered on the left starting at the *EcoRI* linker sequence and the TGA termination codon and putative polyadenylation signal, AATAAA, are indicated. The amino-acid sequence is given below the DNA sequence and its numbering is on the right. METHODS. The *EcoRI* fragment of  $\lambda$ gt11-45W containing *T. ovis* cDNA was cloned in both orientations into phage M13mp18 (ref. 19) for sequencing. Overlapping deletions of the cDNA insert cloned into M13mp18 were obtained by exonuclease III digestion as described by Henikoff<sup>20</sup>. Single-stranded DNA was prepared and both strands were sequenced by the dideoxy chain-termination method<sup>21</sup>.

maintaining the frame of both  $\lambda$ gt11 and pSj10 $\Delta$ Bam7Stop7. The predicted 25.83K peptide agrees with size estimates (26K) of the fusion protein on SDS-PAGE (data not shown), but differs substantially from the size of the native antigens, which may be post-translationally modified. The absence of a consensus translational start signal (ATG) indicated that the cDNA was not a complete copy of the 45W mRNA. Although the extent of the 5' end of the specific mRNA has not been determined, the potential upstream regions do not seem to be necessary for effective vaccination. Indeed, the protective epitope(s) may comprise only a small region of 45W; preliminary sequence data indicate that the non-protective, ~500-base pair 45S clone is a shorter version of 45W, so that further comparative analyses may allow definition of the host-protective epitope(s).

This report describes the first identification of a protective antigen of the taeniid cestodes produced by recombinant DNA methods. Although 100% protection was not achieved, the levels that were attained should enable a significant level of parasite control in the field and could form the basis of a commercial vaccine. □

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## Identification of susceptibility loci for insulin-dependent diabetes mellitus by trans-racial gene mapping

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INSULIN-dependent (type I) diabetes mellitus (IDDM) follows an autoimmune destruction of the insulin-producing  $\beta$ -cells of the pancreas<sup>1,2</sup>. Family and population studies indicate that predisposition is probably polygenic<sup>3</sup>. At least one susceptibility gene lies within the major histocompatibility complex and is closely linked to the genes encoding the class II antigens, HLA-DR and HLA-DQ (refs 3, 4). Fine mapping of susceptibility genes by linkage analysis in families is not feasible because of infrequent recombination (linkage disequilibrium) between the DR and DQ genes. Recombination events in the past, however, have occurred and generated distinct DR-DQ haplotypes, whose frequencies vary between races<sup>4-6</sup>. DNA sequencing and oligonucleotide dot-blot analysis of class II genes from two race-specific haplotypes indicate that susceptibility to IDDM is closely linked to the DQA1 locus and suggest that both the DQB1 (ref. 7) and DQA1 genes contribute to disease predisposition.

The polymorphic HLA-DQA1 and -DQB1 genes encode polypeptides that fold together to form a receptor that specifies T-lymphocyte recognition of self and foreign peptides<sup>8-13</sup>. DNA sequence analysis of DQ and DR genes from Caucasian patients with IDDM indicates that susceptibility to the disease correlates with the amino acid at position 57 of the DQ  $\beta$ -chain, with Asp at this position associated with resistance to IDDM<sup>7,14-16</sup>. An exception to this is that the 'Asp 57-negative' DQ  $\beta$ -chain encoded by DR3-DQw2 haplotypes, which are positively associated with IDDM, is also encoded by DR7-DQw2 haplotypes which are not increased in frequency in Caucasian patients<sup>3,7,17,18</sup>. This difference might arise because DR3 and DR7 haplotypes carry different DQA1 alleles<sup>7,18</sup>.

In black subjects, race-specific DR7 and DR9 haplotypes that are characterized by a unique TaqI restriction fragment-length polymorphism (RFLP) in DQB1, called DQBVIc, have been



TABLE 1 Major histocompatibility complex class II haplotypes and their associations with IDDM in blacks and Caucasians

Locus	DQB1	DQA1	DRB1	DRB4	DRA	Race	IDDM association
Alleles	DQw2	DR7-DQA1	<b>DR7</b>	<b>DRB4</b>	DR4	Caucasian	neutral
	DQw2	DR4-DQA1*	<b>DR7</b>	<b>DRB4</b>	DR4	black	positive
	DQw9	DR4-DQA1	<b>DR9</b>	<b>DRB3</b>	DR4	Caucasian	neutral
	DQw2*	DR4-DQA1	<b>DR9</b>	<b>DRB4</b>	DR4	black	positive

Major histocompatibility complex haplotypes referred to in the text are named according to their DR serological determinants, which correspond to their DRB1 polymorphism (shown in bold type). These haplotypes were determined by a combination of serological, RFLP and ASO dot-blot analysis<sup>6,7,23</sup>. Genes are given in the order in which they exist on chromosome 6.

\* Allelic substitutions that map susceptibility to IDDM to both the DQA1 and DQB1 genes.

TABLE 2 Frequencies of the DR4-DQA1 allele and DR7 haplotypes in black type 1 diabetic patients and control subjects

Specificity	IDDM patients (n=37) n (%)	Controls (n=79) n (%)	Relative risk	95% Confidence limits	P
DR7, DR4-DQA1	7 (18.9)	1 (1.3)	12.9	2.6-64.0	<0.005
DR7, DR7-DQA1	5 (13.5)	7 (8.9)	1.6	0.5-5.0	NS
DR4-DQA1	29 (78.4)	10 (12.7)	23	8.6-61.0	<10 <sup>-6</sup>

DR7, DR4-DQA1 and DR7, DR7-DQA1 refer to the number (%) of subjects with the DR7 antigen and the DR4-DQA1 and DR7-DQA1 alleles, respectively. The total numbers (%) of subjects with the DR4-DQA1 allele are given in the third row. The characteristics of the diabetic patients and control subjects, and the methods for DR serological typing for the RFLP studies and for the statistical analysis have been described previously<sup>6</sup>. All DR9- and DR7-positive control and patient subjects who possessed the DR4-DQA1 allele also possessed the DQBVIc RFLP. The two ASOs used to detect the DR4-DQA1 and DR7-DQA1 alleles were similar to two probes described previously<sup>23</sup>, and had the following sequences, respectively, 5'-TTCCGACAGATTTAGAAGATT-3' and 5'-TCTAAGTCTGTGGAACA-3'. Hybridization and washing conditions for dot blots were as previously described<sup>7</sup>, and washing temperatures were 59 °C and 50 °C for DR4-DQA1 and DR7-DQA1 ASOs, respectively. Using the previously described DQA1/DQA2-specific polymerase chain reaction (PCR) primers<sup>24</sup> and conditions recommended for Taq polymerase<sup>22</sup>, the signal from the dot blots was not satisfactory. Two per cent of the primary PCR product, obtained using the original DQA1/DQA2 primers<sup>24</sup>, was therefore subjected to a second round of Taq polymerase-catalysed amplification<sup>22</sup> using one of the original primers, GH26a (identical to GH26 (ref. 24), except that the restriction-site bases were not included) with a third oligonucleotide, DQA27B, 5'-GTAGAGTTGGAGCGTTA-3' (corresponding to codons 79-83 of the DQA1 gene). NS, Not significant.

described<sup>6</sup>. By contrast with the neutral effects of DR7 and DR9 in Caucasians<sup>3</sup>, both haplotypes are positively associated with IDDM<sup>6</sup>. In the present study, DNA sequences of the class II alleles present on these black haplotypes were analysed and compared with those from non-predisposing Caucasian DR7 and DR9 haplotypes, to identify any class II gene coding differences.

Genes *DRB1*, *DRB4*, *DQA1* and *DQB1* from a black patient, who was homozygous for DR7 and the DQBVIc RFLP, were cloned and sequenced. Analysis of the DNA sequences corresponding to the N-terminal domains of the DRβ1-, DRβ4- and DQB1-chains established that they were identical to those found on Caucasian DR7 haplotypes<sup>7,14,19,20</sup>. The only sequence difference detected was in the DQA1 allele. The black DQA1 allele was identical to the DQA1 allele found on Caucasian DR4 haplotypes (DR4-DQA1; Table 1)<sup>21</sup>.

The *DRB1*, *DRB4*, *DQA1* and *DQB1* alleles from a black patient homozygous for DR9 and the DQBVIc RFLP were also cloned and sequenced. The black DR9 haplotype contained the same *DQB1* allele (DQw2) as the black DR7 haplotype, in contrast to Caucasian DR9 haplotypes, which have the Asp 57-encoding DQw9 allele<sup>7</sup>. The predicted N-terminal amino-acid sequence encoded by the DQA1 allele was the same as that encoded by Caucasian DR9 and black DR7 haplotypes,

DR4-DQA1. The *DRB1* and *DRB4* alleles were identical to those on Caucasian DR9 haplotypes (Table 1)<sup>20</sup>.

To assess the contribution of the DR4-DQA1 allele to IDDM susceptibility, we used allele-specific oligonucleotide (ASO) dot-blot analysis<sup>7,22,23</sup>. Thirty-seven black patients with IDDM and 79 racially matched controls were tested (Table 2). Two ASOs were used, one that hybridizes to only the DR7-DQA1 allele, the other to the DR4-DQA1 allele (Table 2). The analysis revealed that all DR4-positive and DR9-positive patients and control subjects reacted positively with the DR4-DQA1 ASO, but were negative for the DR7-DQA1 ASO. Seven out of 11 DR7-positive patients were positive for the DR4-DQA1 ASO, compared with only 1 out of 8 DR7-positive controls (relative risk of IDDM, 12.9;  $P < 0.005$ ; Table 2). Conversely, 5 out of 11 DR7-positive patients reacted with the DR7-DQA1 ASO compared with 7 out of 8 DR7-positive controls (relative risk, 1.6, not statistically significant; Table 2). One patient, who was DR7-homozygous, was positive for both ASOs. Overall, the relative risk for the DR4-DQA1 allele was 23 (95% confidence limits 8.6-61.0;  $P < 10^{-6}$ ).

The identification of common DR polymorphisms in linkage disequilibrium with a rare combination of DQA1 and DQB1 alleles (Table 1) indicates that susceptibility to IDDM maps



FIG. 1 Arrangement of expressed major histocompatibility complex class II genes, HLA-DR and DQ, on chromosome 6.

centromeric to the DR subregion (Fig. 1). Substitution of the DR4-DQA1 allele for the DR7-DQA1 allele on black DR7 haplotypes correlates with a change in predisposition to IDDM from neutral to positive. Furthermore, substitution of the DQw2-DQB1 allele for the DQw9 allele on black DR9 haplotypes correlates with a change from neutral to positive. These findings indicate that a particular combination of DQA1 and DQB1 alleles is positively associated with IDDM. Although susceptibility may be influenced by other major histocompatibility complex genes, our data suggest that the conformation of the DQ molecules contributes to development of IDDM. □

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## Two functionally different regions in Fos are required for the sequence-specific DNA interaction of the Fos/Jun protein complex

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THE products of the cellular and retroviral *fos* genes associate with other nuclear proteins<sup>1,2</sup>, among them the transcription factor AP1/Jun (see ref. 3 for a review). The Fos/Jun complex binds to a specific symmetrical DNA recognition sequence<sup>3-9</sup> (termed TRE), thus stimulating transcription of the respective gene<sup>3,9-12</sup>. Here, we show that two distinct regions in Fos are required for the formation of a Fos/Jun/TRE complex. These are the leucine zipper<sup>13</sup>, involved in the association with Jun<sup>14</sup>, and a directly adjacent basic region. Specific amino-acid substitutions in this basic, presumably  $\alpha$ -helical, region abolish the interaction of Fos/Jun with the TRE but not the association of the two proteins. The functionally crucial amino acids are located in a region of Fos which is structurally similar to the putative DNA-binding sites in Jun and in the yeast transcriptional activator GCN4 (refs 15 and 16).

As a first step in investigating the role of Fos in the formation of a Fos/Jun/TRE complex we attempted to identify by site-directed mutagenesis those sequences in Fos that are crucial for complex formation. We concentrated this investigation on the

middle region of Fos, which evolutionarily is the most highly conserved<sup>17</sup> and is crucial for the induction of transformation<sup>18</sup> and transactivation<sup>10</sup>. Two long  $\alpha$ -helical regions are predicted in this region (amino acids 139-156 and 163-202)<sup>15</sup>. We generated Fos proteins that carried mutations in the putative second helix which forms a leucine zipper<sup>13</sup> with Jun<sup>14</sup> and various mutants altered in the first helix by exchanging single, pairs or triplets of basic amino acids either with other basic (Arg  $\rightarrow$  Lys; Lys  $\rightarrow$  Arg) or with neutral (Arg, Lys  $\rightarrow$  Gln) amino acids (Fig. 1). We also altered the non-polar sequence Met-Ala-Ala-Ala.

All mutants were analysed for ability to complex with Jun: <sup>35</sup>S-labelled, *in vitro*-translated Jun was incubated with unlabelled Fos, followed by immunoprecipitation with Fos-specific antibodies<sup>14</sup>. As previously shown<sup>14</sup>, binding was either reduced or abolished in several Fos proteins with a mutant leucine-repeat motif (for example, L2, FA183ins, L3-4-5). In contrast, alterations in the basic region did not affect binding to Jun (data not shown).

Next we analysed all mutant Fos proteins in a gel retardation assay using a synthetic oligonucleotide (termed ap1) containing a TRE. Figure 2 (upper panel, lanes 1-4) shows that in the assay conditions used, neither *in vitro*-translated Fos nor Jun shows any significant affinity for the ap1 oligonucleotide. But incubation of the two proteins before addition to the DNA results in a strong shift, indicating that Fos and Jun are binding cooperatively. The specificity of this interaction was demonstrated in competition experiments, in which a >1,000-fold increase in concentration of a random oligonucleotide compared with the specific ap1 oligonucleotide was necessary to abolish the band shift (data not shown). As expected, the ability of the leucine-repeat mutants (L3, L4, L2, L3-4-5, FA183ins) to form a stable Fos/Jun/DNA complex (Fig. 2) correlated well with their ability to associate with Jun<sup>14</sup>: the substitution of Leu 179 (L3), Leu 186 (L4) or Leu 172 (L2) either did not affect (L3) or it decreased (L4; L2) the Fos/Jun/DNA complex formation, whereas the triple leucine mutants L3-4-5 and the insertion mutant FA183ins, in which the orientation of leucine residues on one side of the helix is impaired, did not allow complex formation to any significant extent. These data indicate that the association of Fos and Jun through the leucine zipper is required for their interaction with the AP1 DNA-recognition sequence.

Gel retardation analysis of the other mutant Fos proteins revealed a second functionally unrelated domain in Fos that is involved in Fos/Jun/DNA complex formation. This domain is the basic, presumably  $\alpha$ -helical structure that is directly adjacent to the leucine repeat, as indicated by the reduced ability of mutant D9 and the failure of D3, D15, D19ins, D4 and D16 to

FIG. 1 Structure of the mutant Fos proteins and the parental E300 used in this study. Substituted amino acids are marked by double underlining. Numbers below the E300 sequence designate amino-acid positions in cellular and viral Fos proteins. The E300 Fos protein used in this study is a FBJ/FBR-MuSV hybrid, extending from amino acid 1-313 (ref. 14). The one-letter amino-acid code is used.

METHODS. Oligonucleotide-directed mutagenesis was by the gapped duplex DNA method based on pMa/5-8 and pMc/5-8 vector systems, as previously described<sup>14</sup>. Mutagenic oligonucleotides used were (numbers refer to nucleotide positions in FBR-MuSV<sup>25</sup>): D6: (2,369) 5'CTCTCGGATTGCT-GTTTCACTTGA (2,344); D8: (2,389) 5'GCAGCCAT-CTGATTCTGTTCTCTTCG (2,364); D9: (2,389) 5'GCAG-CCATCTGATTCCGTTCTC (2,368); D13: (2,380) 5'CT-TATTCGGTTCTTTTGTGATTCTCCG (2,353); D15: (2,399) 5'GGCACTTGGATGAAGACAGCTTATTCGTTG (2,369); D16: (2,410) 5'CTCCGATTCTTGACCTGGCTGCAGCC (2,383); D17: (2,421) 5'GTCAGCTCCTTCTTATTCCGGCAC (2,395); D19ins: (2,400)

	Basic region	Leucine repeat
E 300	...KRRIRRRERNKMAAAKCRNRRRELTDTLQAETDQLEDEKSA	LQTEIANLLKEKEKLEF...
	139 150 165 172 179 186 193	
Mutations in basic region		Mutations in leucine repeat
D3	KRRIOQQNQMA...	L2 ...DOVED...
D6	KQOIRRRERNQMA...	L3 ...SAVQT...
D8	KRRIRREQNNQMA...	L4 ...ANALK...
D9	KRRIRRRERNQMA...	L3-4-5 ...SAVQTEIANALKEKEKVEF...
D13	KRRIKKERNKMA...	FA183ins ...TEFAIA...
D15	...NKLSSSKC...	
D19ins	...KMAAAAKC...	
D4	...AAQCCNQQQEL...	
D16	...AARCKNR...	
D17	...RNKKKEL...	

5'CGGCACTTGGCGGCGGCTGCAGCCATC (2,374). Construction of the leucine repeat mutants and of D3 and D4 has been described elsewhere<sup>10,14</sup>.

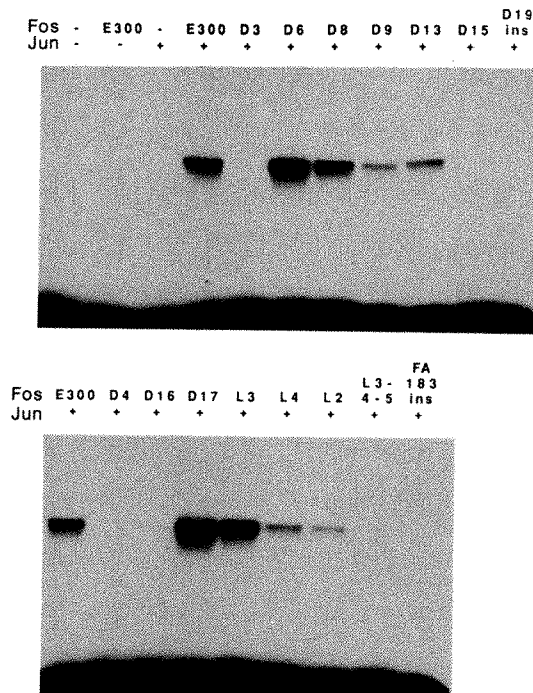


FIG. 2 Gel retardation analysis of complex formation between Fos/Jun and the AP1 DNA-recognition sequence. *In vitro*-translated Fos and Jun proteins were allowed to form a complex as previously described<sup>14</sup> and then incubated with a <sup>32</sup>P-end-labelled double-stranded oligonucleotide (upper strand: 5'AAG-CATGAGTCAGACAC) containing an AP1 recognition sequence. DNA-protein complexes and uncomplexed oligonucleotides were separated on non-denaturing polyacrylamide gels. Protein-DNA complexes were formed in the absence of Fos (E300) or Jun proteins or after incubation of E300 or mutant E300 proteins with Jun. The structure of E300 mutants is shown in Fig. 1. METHODS. E300, *fos* mutants and *c-jun* complementary DNA<sup>26</sup> were transcribed *in vitro* and translated in reticulocyte lysate<sup>14</sup>. Binding reactions were performed by preincubating 5 µl Fos- and 5 µl Jun-containing *in vitro* translation mixtures with 1.5 µg ml<sup>-1</sup> poly(dI-dC) in buffer containing 10 mM HEPES, pH 7.9, 60 mM KCl, 4% Ficoll, 1 mM EDTA and 1 mM dithiothreitol for 60 min on ice. Two femtomoles of double-stranded oligonucleotide <sup>32</sup>P-labelled by polynucleotide kinase were added and incubation continued for 45 min at room temperature. The reaction mixtures were separated on 4% polyacrylamide gels at 10 V per cm<sup>27</sup>.

form a Fos/Jun/DNA complex (Fig. 2). Three aspects of these results deserve particular attention. First, there is no correlation between the elimination of positive charges and the impairment of binding. D6 and D8 (three and two basic amino acids replaced with glutamine, respectively) showed either no change or only a small reduction in their complex-forming potential. In contrast, the mutants having basic for basic amino-acid substitutions (D16) or an altered hydrophobic stretch (D15, D19ins) completely lost their complex-forming potential. Second, the results could indicate that specific interactions with DNA occur in this Fos domain. Although the alteration of certain pairs or triplets of amino acids (D6, D7) did not affect DNA complex-forming ability, the substitution of a single amino acid in D9 or the double mutation in D16 led to a reduction or the abrogation of DNA complex formation. Third, those sequences identified here as being crucial for the formation of a stable Fos/Jun/DNA complex are identical with those most highly conserved between Fos and Jun, namely the putative DNA-binding site in Jun<sup>16</sup> (Fig. 3).

Others have described a cooperative binding of Fos and Jun<sup>19,20</sup> and a crucial function for the leucine-repeat structure in Fos in the formation of a Fos/Jun/TRE complex<sup>21,22</sup>. In one study<sup>21</sup>, a single Fos construct containing a mutation outside the leucine repeat structure (Arg-Arg to Glu-Glu at positions 158-159) was found to have no complex-forming potential, a result differing from ours with mutant D17. This may implicate this region in DNA binding as the structures of both mutant proteins seem to be similar, according to structural predictions using two different algorithms<sup>23,24</sup>.

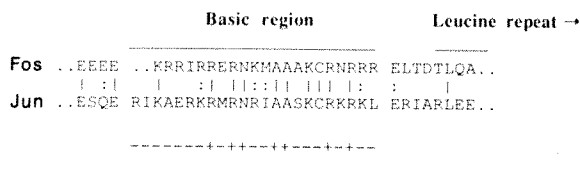


FIG. 3. Region of partial homology between the basic putative  $\alpha$ -helices in Fos and Jun. Vertical lines between Fos and Jun sequences indicate identical amino acids; functionally similar residues are depicted by dots. Amino acids that are conserved (+) or not conserved (-) in the basic regions of Fos, Jun and GCN4 are indicated below the sequences. The region in Fos containing amino acids crucial for DNA binding is marked by a line at the bottom of the figure.

On the basis of the available data, we propose a model in which Fos and Jun form a protein complex through the leucine zipper and each constituent interacts with one half-site of the palindromic DNA sequence. This hypothesis is in agreement with the symmetrical nature of the TRE: similar amino-acid sequences in Fos and Jun would interact with similar DNA sequences. The model is also supported by the observation that the DNA sequences directly flanking the TRE (5'...TGA<sup>c</sup>/GTC...') have no influence on the formation of a stable Fos/Jun/DNA complex (our unpublished results), practically excluding any sequence-specific interaction outside the AP1-recognition sequence. But as direct Fos protein-DNA contacts have been demonstrated by ultraviolet cross-linking experiments<sup>6</sup>, the TRE sequences seem to be the only possible site for a specific interaction of Fos with DNA. A final evaluation of the Fos/Jun/DNA complex will require further mutagenesis, footprint and ultraviolet cross-linking experiments. □

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# The generation of mature T cells requires interaction of the $\alpha\beta$ T-cell receptor with major histocompatibility antigens

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THE T-cell repertoire within an individual is biased to recognize antigen in the context of self major histocompatibility complex (MHC) antigens. This is thought to depend on a process of positive selection during development. Support for this notion has recently been obtained in experiments using transgenic mice bearing genes for T-cell receptors (TCR) of defined specificity: T cells expressing the introduced genes form the main part of the mature T-cell population only in mice that express the appropriate MHC product<sup>1,2</sup>. We have now extended these observations using TCR transgenic mice homozygous for the severe combined immunodeficiency (SCID) mutation which are defective in the rearrangement of both TCR and immunoglobulin genes. In this case mature thymocytes develop only in transgenic mice that express the MHC product which restricts the specificity of the transgenic TCR. This shows that the interaction of the  $\alpha\beta$  TCR with thymic MHC antigen is essential for the development of mature T cells. Furthermore, the peripheral lymph nodes of such mice are underdeveloped, suggesting that the peripheral expansion of mature T cells may require interactions with other lymphocytes expressing a range of receptors.

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To produce mice which have only a very limited choice of TCRs we introduced the SCID mutation<sup>3</sup> into our  $\alpha\beta$  TCR transgenic mice. Mice homozygous for this mutation are grossly defective in productive rearrangements of immunoglobulin (Ig) and TCR genes<sup>4</sup>. We crossed the  $\alpha\beta$  TCR transgenic mouse line TG71<sup>5</sup> with C.B-17/Icr SCID mice and transgenic mice of the F<sub>1</sub> generation were further backcrossed to SCID mice. Mice were typed SCID/SCID by quantitating serum immunoglobulin levels. Because the TG71 line is H-2<sup>b</sup> and the C.B-17/Icr line is H-2<sup>d</sup> homozygous, H-2<sup>b/d</sup> heterozygous as well as H-2<sup>d</sup> homozygous  $\alpha\beta$ -transgenic SCID mice were obtained in the backcrosses. In initial experiments we compared the thymus of the SCID and transgenic SCID mice: the thymus of the former contained on average  $2 \times 10^6$  thymocytes whereas that of the latter contained more than  $5 \times 10^7$  thymocytes irrespective of the MHC haplotype. Although thymocytes from both types of mice were Thy-1-positive, only those of the transgenic SCID mice expressed CD4 and CD8 molecules (Fig. 1). (The weak staining of thymocytes from SCID mice with CD4 antibodies was probably due to nonspecific binding of this conjugate to CD4<sup>8</sup> lymphocytes because it was not observed with another CD4 antibody.) These results imply that the maturation of CD4<sup>8</sup> thymocytes from CD4<sup>8</sup> precursors<sup>6</sup> requires the productive rearrangement of TCR DNA segments. Crosses with mice expressing only the  $\alpha$ - or  $\beta$ -transgene are required to determine which TCR gene segments are necessary and sufficient for this step. These data indicate that the only defect of SCID mice is the defective rearrangement mechanism.

In further experiments we compared the cellular composition of the thymus from female H-2<sup>b/d</sup> and H-2<sup>d</sup>  $\alpha\beta$ -transgenic SCID mice. Because the transgenic TCR would essentially be the only receptor which could be used by the animals, one might expect that the H-2<sup>b/d</sup>, but not the H-2<sup>d</sup> animals, would contain CD4<sup>8</sup> thymocytes, if an interaction of the TCR with MHC antigens was essential for the generation of mature T cells. Double staining with CD4 and CD8 antibodies showed that this was indeed the case: the thymus of the H-2<sup>b/d</sup> transgenic SCID mice contained CD4<sup>8</sup>, CD4<sup>8</sup>, CD4<sup>8</sup> but not CD4<sup>8</sup> cells whereas that of the H-2<sup>d</sup> transgenic SCID mice contained

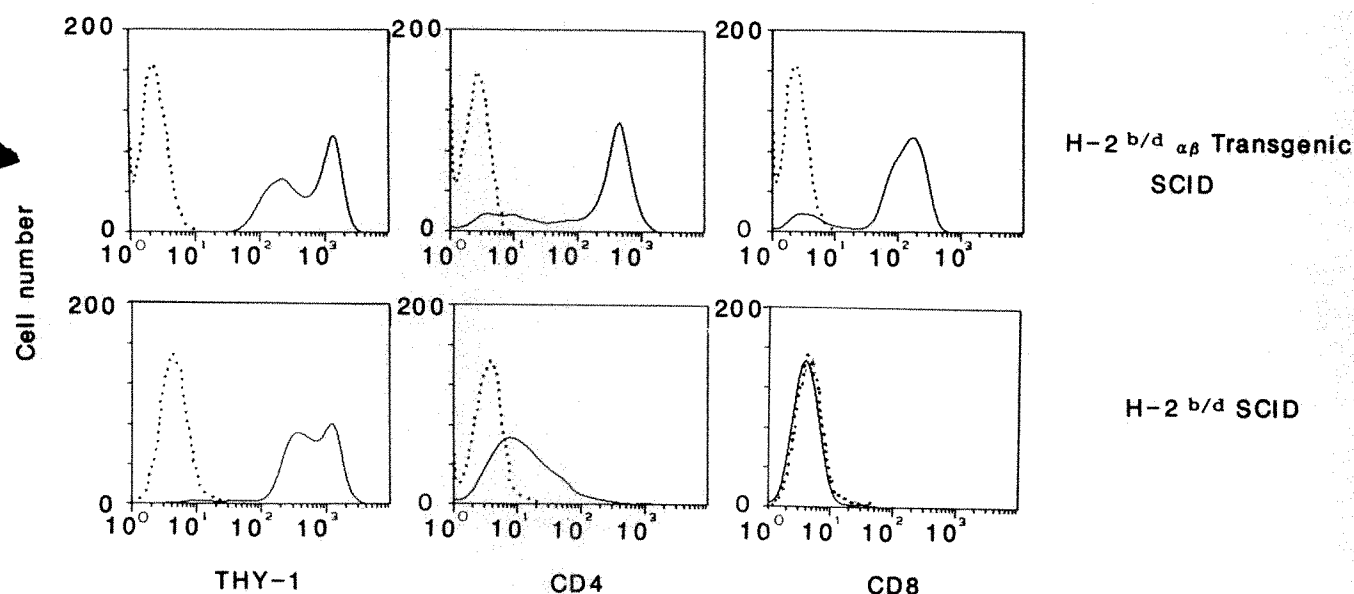


FIG. 1 Generation of CD4<sup>+</sup> and CD8<sup>+</sup> thymocytes in  $\alpha\beta$ -transgenic SCID mice. Thymocytes from female H-2<sup>b/d</sup>  $\alpha\beta$ -transgenic SCID (upper panels) and H-2<sup>b/d</sup> SCID mice (lower panels) were analysed for the expression of Thy-1, CD4 and CD8. Cell numbers are represented on the vertical axis and fluorescence, on a logarithmic scale, on the horizontal axis. The low level of CD4 staining of thymocytes from SCID mice is probably due to the non-specific binding of this reagent to large lymphoblasts which predominate

in the SCID thymus.

**METHODS.** Single staining of cells involved either incubation with a directly labelled antibody (CD4 or CD8) or a first stage antibody (anti-Thy-1, F23.1 or T3.70) followed by a second stage FITC conjugated (Fab)<sub>2</sub> sheep antimouse immunoglobulin (FITC-SαMlg, Silenus). All incubations were for 20 min on ice with two washes between the steps and before analysis.

CD4<sup>-</sup> and CD4<sup>+</sup> but neither CD4<sup>-</sup> nor CD4<sup>+</sup> cells (Fig. 2). Staining with the F23.1 (not shown) and T3.70 monoclonal antibodies detecting the transgenic  $\beta$  and  $\alpha$ TCR chains, respectively<sup>7</sup> indicated that in the various transgenic SCID mice practically all thymocytes expressed the transgenic receptor. The H-2<sup>b/d</sup>  $\alpha\beta$ -transgenic SCID mice contained two populations of cells expressing high levels of the transgenic  $\alpha\beta$ -receptor, namely CD4<sup>-</sup> and CD4<sup>+</sup> thymocytes. The only population with a high density of TCRs was the CD4<sup>-</sup> population in H-2<sup>d</sup> transgenic SCID mice. Both types of animals contained CD4<sup>+</sup> thymocytes expressing lower levels of the transgenic TCR<sup>1,2</sup>. Similar results were obtained in additional two H-2<sup>b/d</sup> and four H-2<sup>d</sup>  $\alpha\beta$ -transgenic SCID mice. Thus, by contrast with  $\alpha\beta$ -transgenic H-2<sup>d</sup> mice, the thymus of H-2<sup>d</sup>  $\alpha\beta$ -transgenic SCID mice does not contain significant numbers of mature CD4<sup>+</sup> or CD4<sup>+</sup> thymocytes. This indicates that the generation of mature, single, positive thymocytes requires a TCR-

MHC interaction and that positive selection does not simply reflect the preferential expansion of some mature T cells.

The size of the thymuses in the  $\alpha\beta$ -transgenic SCID mice was almost normal whereas the peripheral lymphoid tissue in all transgenic SCID mice was clearly underdeveloped: the lymph nodes of these mice contained on average only 10 % of lymphocytes when compared with  $\alpha\beta$ -transgenic mice kept under identical conditions. Double staining with various antibodies showed that the lymphocytes from only H-2<sup>b/d</sup> mice contained significant numbers of CD4<sup>-</sup> T cells which stained with both F23.1 and T3.70 antibodies. H-2<sup>d</sup> transgenic SCID mice contained very few CD4<sup>+</sup> cells and most of these did not express the transgenic  $\alpha$ -chain. Both types of animals contained over 20 % of CD4<sup>-</sup> lymphocytes expressing the transgenic receptor. Finally, in H-2<sup>b/d</sup> as well as H-2<sup>d/d</sup> mice, ~30% of CD4<sup>+</sup> T cells were detected which expressed mostly the transgenic  $\beta$ - but not the transgenic  $\alpha$ -chain (Fig. 3). Immunoglobulin-

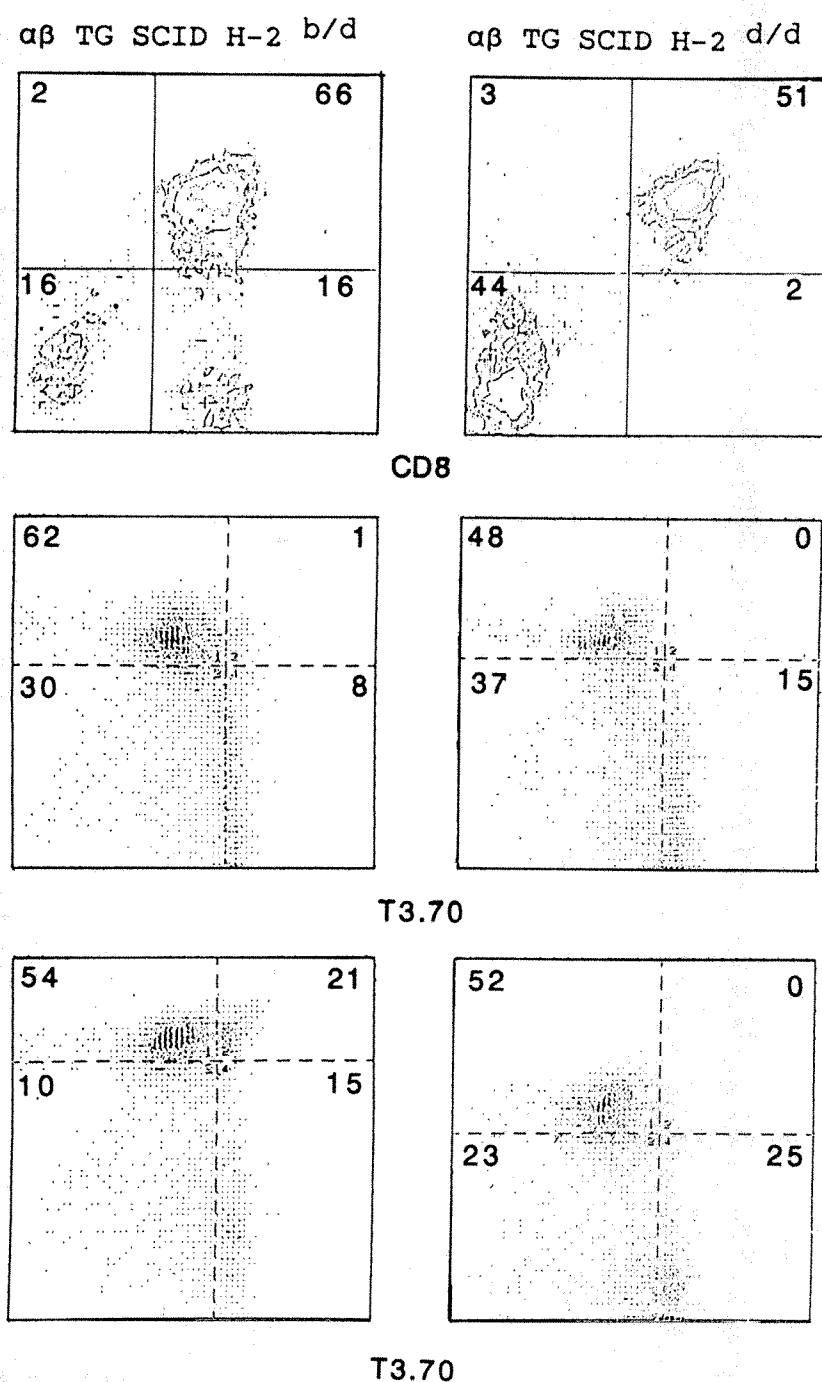


FIG. 2 Thymocyte subpopulations defined by CD4, CD8 and T3.70 antibodies. Thymocytes from female H-2<sup>b/d</sup> and H-2<sup>d/d</sup>  $\alpha\beta$ -transgenic SCID mice were double stained with CD4/CD8, CD4/T3.70 and CD8/T3.70 antibodies. Numbers in quadrants give the percentage of cells in the quadrants. The conditions of staining and the antibodies have been described previously<sup>1,2</sup>.

**METHODS.** Double labelling of cells involved a first stage incubation with either F23.1 or T3.70 followed by a second incubation with FITC-SaMlg. Potentially free sites for binding of immunoglobulin on the fluoresceinated reagent were saturated by a third incubation with normal mouse serum (2% v/v). The cells were then incubated with either PE-anti CD4 or biotinylated anti-CD8 antibodies. Cells incubated with the latter required a final stage of PE-streptavidin (Becton Dickinson). All incubations were for 20 min on ice with two washes between each step and before analysis. All analyses were performed on the FACScan flow cytometer (Becton Dickinson). Single staining required the analysis of 5,000 cells per sample; double staining required 10,000 cells per sample. Dead cells were excluded from the analyses on the basis of low, forward and sideways, light-scattering properties. All graphs were produced from the use of FACScan research software except the contour plots which utilized a CONSORT-30 program.

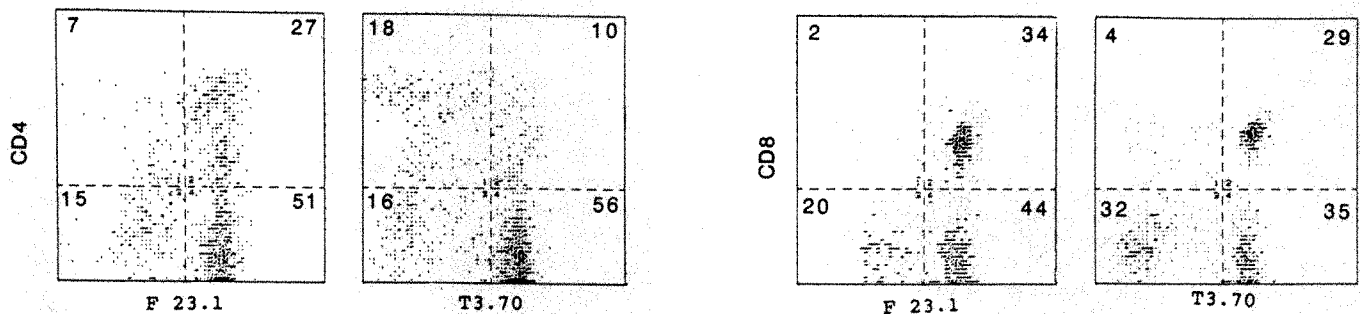
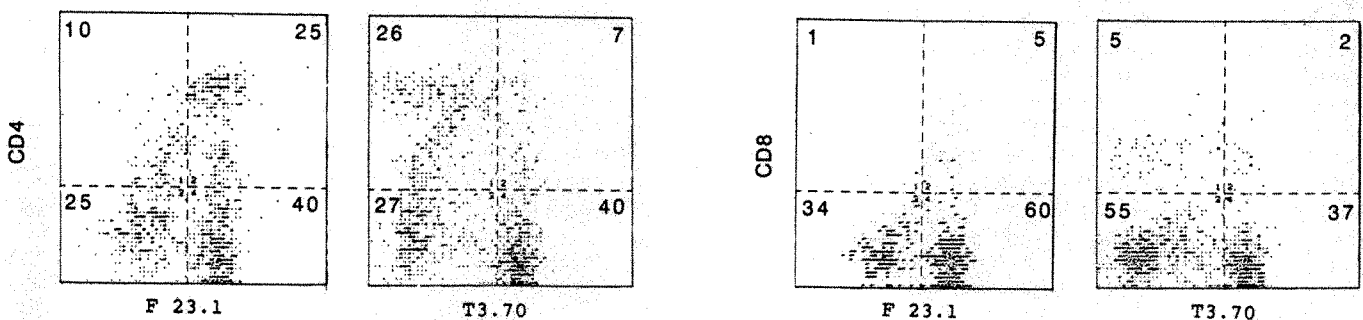
$\alpha\beta$  TG SCID H-2<sup>b/d</sup> $\alpha\beta$  TG SCID H-2<sup>d/d</sup>

FIG 3 Lymphocyte subpopulations defined by CD4, CD8, F23.1, and T3.70 antibodies. Lymphocytes from female H-2<sup>b/d</sup> and H-2<sup>d</sup>  $\alpha\beta$ -transgenic SCID mice were double stained with CD4/F23.1, CD4/T3.70, CD8/F23.1 and

CD8/T3.70 antibodies. Numbers in the quadrants give the percentage of lymphocytes in the quadrants. Conditions of staining and the antibodies have been described previously<sup>1,2</sup>.

positive cells were not detected in any of the lymphocytes. These data indicate that productive rearrangements are made infrequently in SCID mice. In non-transgenic SCID mice this is not often observed because the formation of a receptor-bearing lymphocyte requires several consecutive productive rearrangements in a given lineage. In the transgenic SCID mice, productive  $\alpha$ -gene rearrangements are much easier to detect because the endogenous  $\alpha$ -chain can pair with the transgenic  $\beta$ -chain, the formation of which would require two productive rearrangements in non-transgenic SCID mice. This is probably the reason why we see T cells expressing endogenous  $\alpha$ TCR chains but cannot detect significant numbers of B cells, which again would require multiple productive rearrangements of endogenous immunoglobulin receptor gene segments. Because we cannot detect significant numbers of CD4<sup>+</sup>8<sup>+</sup> or CD4<sup>+</sup>8<sup>+</sup> thymocytes expressing endogenous  $\alpha$ -chains in the H-2<sup>d</sup>  $\alpha\beta$ -transgenic SCID mice, we conclude that very few cells of these phenotypes are generated. But when they are and express a selectable receptor, they leave the thymus and accumulate in the peripheral lymphoid tissue.

This is in marked contrast to CD4<sup>+</sup>8<sup>+</sup> cells expressing high levels of TCR. These cells are at least as frequent in the thymus and periphery in H-2<sup>d</sup> as in H-2<sup>b/d</sup>  $\alpha\beta$ -transgenic SCID mice even though they express the same  $\alpha\beta$ -transgenic receptor. Thus the generation of  $\alpha\beta$  TCR positive CD4<sup>+</sup>8<sup>+</sup> peripheral T cells does not require positive selection by restricting D<sup>b</sup> MHC antigens.

As  $\alpha\beta$ -T-cells are produced in the SCID mice in the absence

of detectable  $\gamma\delta$  cells it seems that the latter do not play an essential part in the development of CD4<sup>+</sup>8<sup>+</sup>, CD4<sup>+</sup>8<sup>+</sup> or CD4<sup>+</sup>8<sup>+</sup> T cells. This does not exclude  $\gamma\delta$ -cells from being essential for the formation of cells expressing rearranged TCR  $\beta$ -genes because our mice contain already rearranged  $\beta$ -genes.

Finally, the underdeveloped tissue of secondary lymphoid organs in H-2<sup>b/d</sup>  $\alpha\beta$ -transgenic SCID mice deserves some comment: in these mice CD4<sup>+</sup>8<sup>+</sup> cells are continuously positively selected in thymus yet the absolute numbers of peripheral lymphocytes are small. This suggests that the formation of peripheral lymphoid tissue requires immunoglobulin positive lymphocytes as well as CD4<sup>+</sup>8<sup>+</sup> T cells with a diverse range of receptors. Thus, antigenic stimulation and interaction of various lymphocyte subsets may be an important factor regulating the size of lymphoid tissue. □

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# Phenol stabilizes more helix in a new symmetrical zinc insulin hexamer

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SINCE insulin was first shown by Scott<sup>1</sup> to crystallize in the presence of zinc ions in 1934, a variety of Zn-containing insulin crystals have been grown<sup>2,3</sup>. The structures of insulin in the related rhombohedral crystals of 2Zn-insulin and 4Zn-insulin have been solved<sup>4,5</sup> and reveal that the molecule is a hexamer, organized as three dimers, each containing a 2-fold symmetry axis and held together by Zn ions. In 2Zn-insulin the hexamer is nearly symmetrical with the two axial Zn ions and the two molecules of the dimer related closely by a local 2-fold axis. But in 4Zn-insulin the two molecules in the dimer differ remarkably, creating an asymmetric 4Zn-hexamer in which one trimer is essentially equivalent to that in 2Zn-insulin and the other is different by virtue of an additional stretch of N-terminal helix between residues B1 and B8 (refs 6, 7). We report here the structure of a new symmetrical hexamer, in which all six molecules have the B1-B8 helix seen in 4Zn-insulin. Phenol molecules, found bonding specifically to each molecule, evidently stabilize this new helical conformation.

The existence of a symmetrical hexamer in which all six insulin molecules have a helical conformation from B1-B20 has been predicted<sup>8,9</sup> and the capacity of phenol to promote this helix in solution has been observed<sup>10</sup>. Monoclinic crystals of hexameric insulin grown in the presence of phenol, added as a preservative, have long been known<sup>2</sup> and it was from these that the new hexamer was discovered. The space group is  $P2_1$  and the crystals contain a complete hexamer in the asymmetric unit (Table 1). The chemical analysis of these crystals showed that they contained two Zn ions per insulin hexamer<sup>2</sup>, which suggested they were related in a simple manner to that of the rhombohedral 2Zn-insulin hexamer.

A full X-ray analysis of these crystals was originally under-

taken to investigate the loss of 3-fold symmetry in the hexamer and its effect upon the conformation of each of the monomers. Early experiments to prepare heavy-atom derivatives had limited success (J. Dargay, D. Hodgkin and G.G.D., unpublished observations). The structure was determined by molecular replacement, using the accurate 2Zn-insulin coordinates<sup>11</sup> as an initial model, and refined<sup>12,13</sup>. During the refinement it became clear that the hexamer in the crystal was different from the assumed 2Zn-insulin model, primarily at residues B1-B8. Inspection of the difference Fourier maps revealed electron density tracing a helix from B9-B1 in all six molecules, reminiscent of that seen in one trimer of the rhombohedral 4Zn-insulin hexamer (see Fig. 1). Consequently, a new hexamer was constructed using six monomers with properly oriented  $\alpha$ -helices from B1-B20.

As the refinement converged, a large region of strong electron density, obviously not water, was identified next to each of the B5 His side-chains. Continued refinement with the 1.8 Å data resulted in a map in which the phenol molecules were mostly well resolved and the positions of their hydroxyl groups clearly indicated (see Fig. 2). Each of the six phenol molecules donates a hydrogen bond to the A6 Cys carbonyl oxygen and accepts a hydrogen bond from the All Cys amide nitrogen. The phenol molecules sit in the cavity created by the A-chain residues from one dimer and the B1-B8 helical structure from the adjacent dimer. This allows the B5 His to link the dimers through van der Waal's contacts with the phenol molecule; in 4Zn-insulin, the same effect is achieved by the off-axial Zn ions. As shown in Fig. 1 there is a clear progression in the amount of helix present between residues B1 and B20 in the hexamers in rhombohedral 2Zn-insulin, 4Zn-insulin and monoclinic insulin crystals. These hexamers have been designated respectively 'T<sub>6</sub>', 'T<sub>3</sub>/R<sub>3</sub>' and 'R<sub>6</sub>', as described in Fig. 1.

The finding that phenol binds and stabilizes helix at B1-B8 in the insulin hexamer is a reminder of how complex interactions between ligands and proteins can be. It also raises the question of how the Zn-insulin hexamers stored in the  $\beta$ -cell are constructed: the high concentrations of peptides, Ca<sup>2+</sup>, Zn<sup>2+</sup> and counterions could all influence the conformation at B1-B8. Moreover, the switch from extended sheet to helix is reminiscent of structural transitions observed in signalling and allosteric mechanisms, often associated with Zn<sup>2+</sup> or Ca<sup>2+</sup> (ref. 14).

The similarity between phenol and tyrosine raises the possibility that a tyrosyl side-chain from the insulin receptor stabilizes the B-chain N-terminal helix in the course of generating a signal

TABLE 1 Crystallographic and structural data

Hexamer type	Space group	No. molecules per asymmetric unit	Cell parameters	$R_{\text{cryst}}^*$	Resolution	Approximate point symmetry in hexamer	No. Zn sites	Chemical species associated with Zn	Limits of B-helix	
2Zn-insulin (T <sub>6</sub> )	R3	2	$a_h = 82.5$ , $b_h = 82.5$ , $c_h = 34.0$ , $\gamma = 120^\circ$	0.154	1.5 Å	32	2	H <sub>2</sub> O	B9-B20	M1‡
4Zn-insulin (T <sub>3</sub> /R <sub>3</sub> )	R3	2	$a_h = 80.7$ , $b_h = 80.7$ , $c_h = 37.6$ , $\gamma = 120^\circ$	0.18	1.5 Å	3	5	Cl, H <sub>2</sub> O	B9-B20	M2
2Zn-insulin phenol (R <sub>6</sub> )	P2 <sub>1</sub>	6	$a = 61.36$ , $b = 61.71$ , $c = 47.95$ , $\beta = 110.8$	0.22	1.8 Å†	32	2	Cl, H <sub>2</sub> O	B1-B20	M1
									B1-B20	M2
									B1-B20	M3
									B1-B20	M4
									B1-B20	M5
									B1-B20	M6

\*  $R_{\text{cryst}} = \sum ||F_o| - |F_c|| / \sum |F_o|$  where  $|F_o|$  and  $|F_c|$  are the observed and calculated structure amplitudes respectively (all data included).

† Diffraction data collected on a diffractometer extend to 2.5 Å (15,785 reflections); data collected with synchrotron radiation extend to 1.8 Å (30,841 reflections). The molecular replacement and initial refinement calculations were carried out on the diffractometer data. Refinement of the atomic positions and thermal parameters were carried out on a combined diffractometer and synchrotron radiation data set ( $R_{\text{merge}} = 0.06$ ). The usual Fourier and fast Fourier least squares minimization methods were used in which the calculated shifts were restrained by the requirements of peptide geometry<sup>12,13</sup>. All 2,430 protein atoms in the asymmetric unit have been located and refined; the six phenol molecules and 421 water molecules have also been identified and included in the refinement calculations. The  $R_{\text{cryst}}$  on all data is 0.22. The r.m.s. deviation from ideal bond lengths is 0.03 Å; accuracy of the well defined atomic positions is estimated to be about 0.2 Å. The atomic parameters will be deposited in the Brookhaven Data Bank.

‡ M1-M6 represent the insulin molecules in the asymmetric unit.

FIG. 1 Arrangement of the three monomers around the upper (a) and lower (b) Zn ions, and the complete hexamer structures (c) in the 2Zn- and 4Zn-insulin crystals and the 2Zn-insulin (phenol) crystals. C $\alpha$  positions are shown together with B5 and B10 histidyl side chains involved in Zn coordination. The A-chain is drawn with open bonds, the B-chain B9-B30 is drawn as a single line and the B-chain residues B1-B8 are drawn in heavy lines. The phenol molecules and the off-axial Zn sites in 4Zn-insulin are also shown. The stoichiometry of Zn in 4Zn-insulin is not well defined: when the off-axial sites are fully occupied in molecule 2 (b), there are 4Zn ions per hexamer, but two populations, with alternative B10 His conformations also occur. One of these has Zn bound on the axial site (to B10 His); the other has Zn bound at the off-axial site (B5 His and B10 His). The conformation that does not bind phenol is analogous to an allosteric T state; the extended conformation at B1-B8 is thus called T. By the same analogy, R represents the structure with helix at B1-B8 that binds phenol.

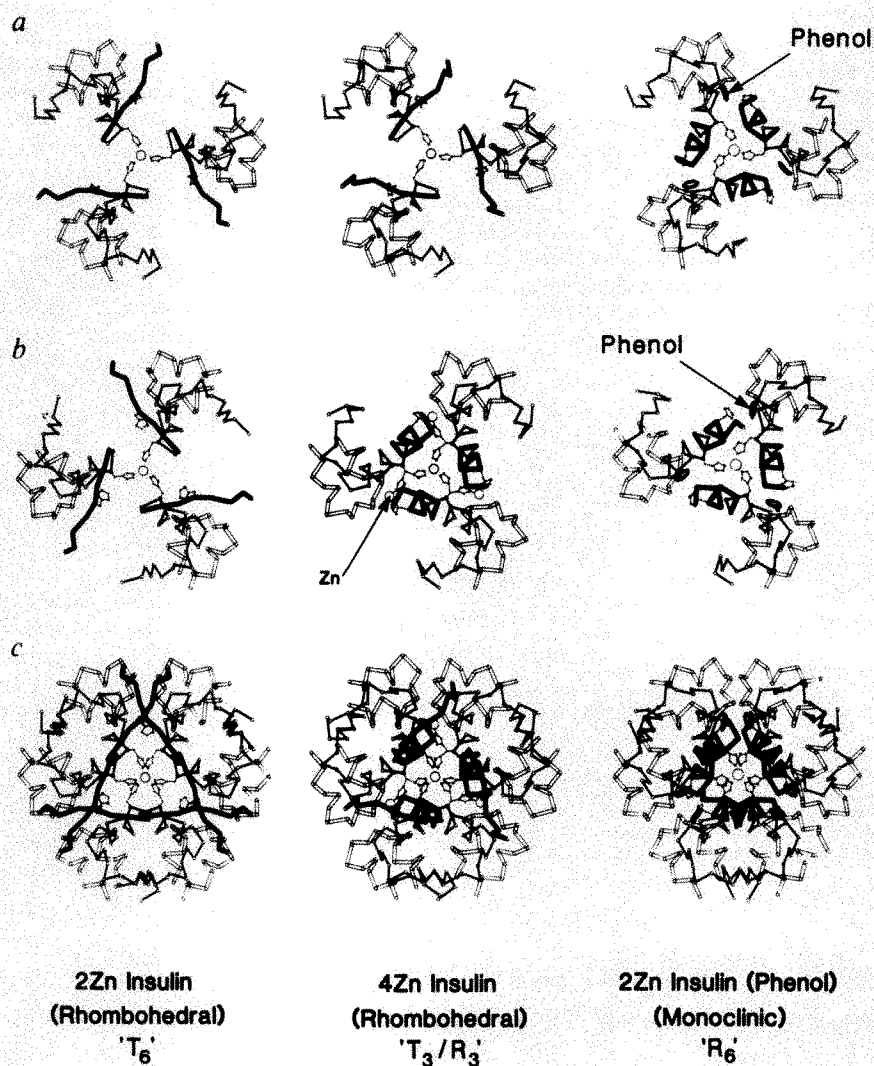
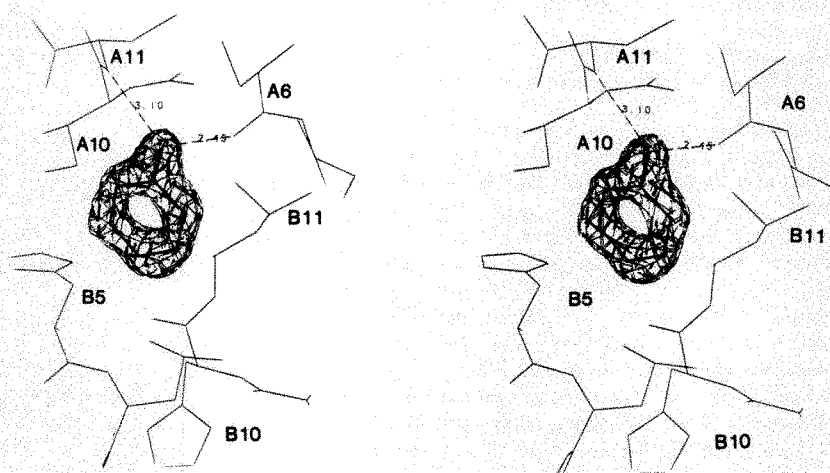


FIG. 2 The electron density of one of the phenol molecules. The OH group is hydrogen-bonded to the carbonyl oxygen and amide nitrogen of the A6 and A11 cysteines respectively, linked in a disulphide bond. The phenol makes van der Waal's contacts with the dimer-dimer interface (Fig. 1). The imidazole B5 group makes a roughly perpendicular approach of 3.4 Å to the phenol molecule. It belongs to an insulin molecule from one dimer; the A-chain residues surrounding the other surfaces of the phenol are from the insulin molecule in the adjacent dimer. Other contacts are not shown.



after hormone binding. Independent studies have shown that phenol binding greatly reduces exchange with solution by the  $\text{Zn}^{2+}$  ions trapped by the B1-B8 helix extension (A. Wollmer and M. Dunn, personal communication). Several commonly used insulin preparations use phenol or similar molecules as a preservative<sup>15</sup>. The protamine (NPH) insulin, for example, contains phenol or cresol at concentrations (0.1–0.25%) high enough to induce the helix structure at B1-B8 observed in the monoclinic insulin crystals. Indeed, the 'R<sub>6</sub>' hexamer with six bound *m*-cresol molecules has been recently identified in NPH insulin crystals, helping to explain their extra stability (F. Korber, personal communication). It is conceivable that other phenol-like molecules could also be used to enhance the stability of the hexamer in solution, leading to a range of insulin preparations of increased stability. □

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## Biosynthesis of cadmium sulphide quantum semiconductor crystallites

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**NANOMETRE-SCALE semiconductor quantum crystallites exhibit size-dependent and discrete excited electronic states which occur at energies higher than the band gap of the corresponding bulk solid<sup>1–4</sup>. These crystallites are too small to have continuous energy bands, even though a bulk crystal structure is present. The onset of such quantum properties sets a fundamental limit to device miniaturization in microelectronics<sup>5</sup>. Structures with either one, two or all three dimensions on the nanometer scale are of particular interest in solid state physics<sup>6</sup>. We report here our discovery of the biosynthesis of quantum crystallites in yeasts *Candida glabrata* and *Schizosaccharomyces pombe*, cultured in the presence of cadmium salts. Short chelating peptides of general structure ( $\gamma$ -Glu-Cys)<sub>n</sub>-Gly control the nucleation and growth of CdS crystallites to peptide-capped intracellular particles of diameter 20 Å. These quantum CdS crystallites are more monodisperse than CdS particles synthesized chemically. X-ray data indicate that, at this small size, the CdS structure differs from that of bulk CdS and tends towards a six-coordinate rock-salt structure.**

*C. glabrata* and *S. pombe* respond to Cd salts by synthesizing  $\gamma$ -glutamyl peptides<sup>7,8</sup>. Cytoplasmic cadmium ions are sequestered within complexes containing these  $\gamma$ -Glu peptides<sup>7,9</sup>. Related metal- $\gamma$ -peptide complexes are found in metal-exposed plants and *Euglena gracilis*<sup>10–12</sup>. The metal-peptide complexes readily incorporate sulphide ions arising from a Cd-mediated enhancement of cellular sulphide generation<sup>13–15</sup>. Typical isolates of such cytoplasmic metal-thiolate-peptide complexes can be resolved by gel filtration into clusters varying in sulphide content<sup>14</sup>. The Stokes' radius of the metal-thiolate-peptide complex increases in direct proportion to the sulphide/cadmium ratio. Thus, complex size seems to be dictated by the magnitude of the metal-induced sulphide response.

Transmission electron microscopy (TEM) of the Cd- $\gamma$ -Glu peptide complex (S/Cd=0.7) from *C. glabrata* shows dense aggregates of particles, each having diameters of  $20 \pm 3$  Å and a Stokes' radius of  $\sim 16$  Å. Similar complexes from *S. pombe* (S/Cd=0.6) are  $\sim 18$  Å in diameter. In both cases, the powder X-ray pattern of lyophilized material shows one sharp low-angle peak (Fig. 1), indicating a dense aggregate of near monodisperse, spherical, homogeneous particles. The particle diameters obtained by X-ray diffraction are consistent with those from the electron microscope.

The Bragg diffraction patterns show clear differences between the biogenic crystallites and the bulk solid. The Cd-S bond is on average shorter in the crystallite by about 3–6%. The diffraction pattern from the *S. pombe* complex is accounted for best by the six-coordinate rock-salt structure; a structure previously reported for CdS only at high pressure<sup>16</sup>. The *C. glabrata* complex gives rise to a diffraction pattern consistent with a structure which is intermediate between rock-salt and the naturally occurring zinc-blende four-coordinate structure. Both show an 8 Å coherence length, corresponding to a short network of about four identical Cd-S bonds; surface-bonding changes may contribute to such short lengths in small particles. TEM internal lattice images are not on average observed because of this short

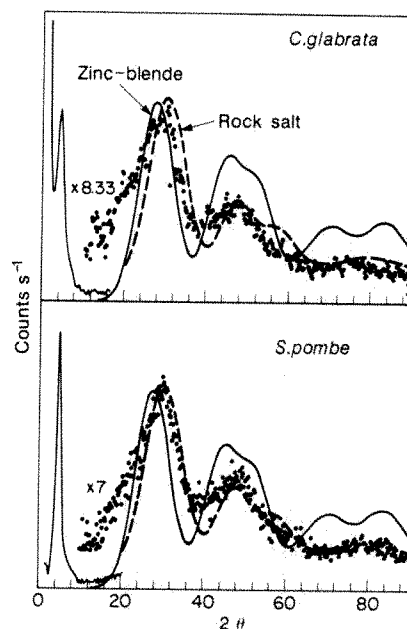


FIG. 1 1.54 Å powder X-ray patterns of desalted lyophilized Cd- $\gamma$ -Glu peptide complexes from *C. glabrata* (top) and *S. pombe* (bottom). The samples were purified by a combination of ion-exchange and gel-filtration chromatography as described previously<sup>9,15</sup>. The samples were desalted by chromatography on Sephadex G-25 equilibrated and eluted with deionized water. Desalted samples were dried in X-ray tubes under vacuum. Solid and dashed lines, expected patterns for zinc-blende and rock-salt lattices, respectively, with an 8 Å coherence length. The sharp peak at low angle ( $4^\circ$ ) represents particle spacing in the dense isolate. The nearest-neighbour distances are 22 Å and 20 Å for isolates from *C. glabrata* and *S. pombe* respectively.



coherence length. The  $\gamma$ -glutamyl peptide coating of the *C. glabrata* 20-Å crystallites consists of approximately 30 peptides of predominantly  $n_2$  and  $n_2$  desGly. The cysteinyl thiolates in the peptides are surface ligands to the core of  $\sim 85$  CdS units.

Both yeasts also produce larger, extracellular CdS particles of uncharacterized coating, whose high apparent molecular weight is indicated by sedimentation at 40,000g and elution in the excluded volume on Sepharose 6B. Particles of diameter  $29 \pm 5$  Å, some of which show zinc-blende internal lattice images (Fig. 2), are shown by electron microscopy. The X-ray Bragg pattern also reveals a zinc-blende structure with an 8 Å coherence length.

The optical band gap of synthetic CdS quantum crystallites increases with decreasing diameter, in agreement with theory<sup>17</sup>. Similarly, the lowest excited state of the 20 Å intracellular particles occurs between 300 and 315 nm (Fig. 3), almost 1.5 eV above the bulk band gap. Smaller sulphide-deficient complexes in *S. pombe* have transitions shifted to higher energy. This spectral shift<sup>14</sup> reflects the size variation of the CdS core. Significantly, extracellular zinc-blende CdS particles of diameter  $\sim 29$  Å have a 365 nm transition (Fig. 3) which is similar to that of synthetic CdS crystallites of comparable diameter (Fig. 3, inset).

Approaches to synthesizing quantum crystallite colloids of CdS have used microstructured environments and/or polymeric surfactants to prevent irreversible precipitation of bulk solid<sup>18-23</sup>. Quantum crystallites previously isolated and purified, have been capped with small organic groups<sup>24</sup>, or with polymeric sodium hexametaphosphate<sup>20</sup>. Only zinc-blende structures have been reported. The narrow, low-angle X-ray peaks (Fig. 1), and the narrow optical transition in the *C. glabrata* particle, imply that these biogenic, short-peptide-capped CdS crystallites are more monodisperse than typical synthetic particles.

Preliminary experiments *in vitro* indicate that these peptides are effective in terminating the growth of crystallites of a specific size. The intracellular crystallites exhibit properties common to small synthetic semiconductor particles such as luminescence at  $\sim 460$  nm and photo-induced electron transfer to methyl viologen. At physiological pH the peptide-coated particles are stable; at pH 3.5, accretion and growth readily occur.

We report here the first demonstration of the biological synthesis of quantum semiconductor crystallites of CdS. Because

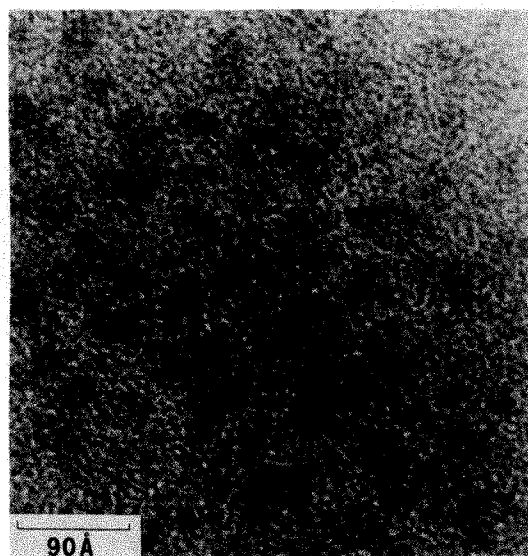


FIG. 2 High-resolution, axial bright-field transmission electron micrograph taken near the Scherzer focus of the *S. pombe* extracellular CdS particle. The sample was dissolved in an aqueous ionic surfactant solution and dried on a thin carbon substrate. Some crystallites show faint images of internal lattice structure when a (111) axis lies in the substrate plane.

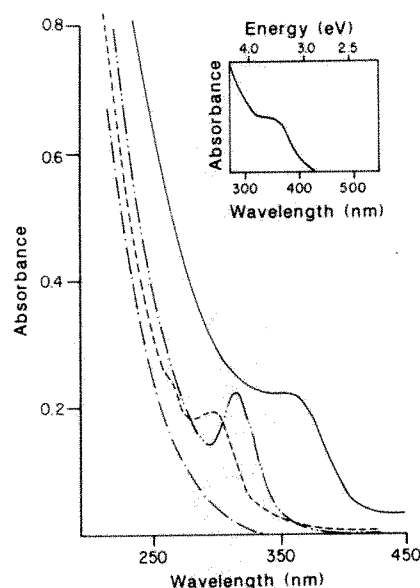


FIG. 3 Optical absorption spectra of the CdS complexes. The samples include peptide-bound CdS particles from *C. glabrata* (—), *S. pombe* (---) and the extracellular CdS particle from *S. pombe* (-·-·-). The samples contain 4, 8 and 20  $\mu\text{g}$  Cd<sup>2+</sup> per ml, respectively. The extracellular CdS complex was isolated by sedimentation at 40,000g and followed by washings. The precipitate was resuspended in 20 mM potassium phosphate, pH 6.5, and concentrated by ultrafiltration (Amicon PM30) followed by filtration (0.2  $\mu\text{m}$  filter). The 0.2  $\mu\text{m}$  filtrate was precipitated with 30 mM Mg<sup>2+</sup> and the final precipitate was resuspended in water and analysed. The (—) line represents the absorption spectrum of *C. glabrata* CdS peptide complex acidified to pH 1.5 to remove H<sub>2</sub>S and re-neutralized to pH 7. The inset is the optical absorption spectra of a chemically prepared 30-Å-diameter zinc-blende CdS crystallite described previously<sup>4</sup>.

the dissociation constant of crystalline CdS is extremely small, bioformation of solid CdS sequesters and thus detoxifies intracellular Cd ions. Electron-dense CdS granules have also been observed on the cell surface of the bacterium *Klebsiella aerogenes*<sup>25</sup>.

Quantum, metal sulphide crystallites have optical absorption, photosynthetic and electron transfer properties that are size-tunable<sup>1,3</sup>. The peptides used by these yeasts occur quite commonly in nature. It is conceivable that in some organisms metal sulphide crystallites have a physiological role other than detoxification. □

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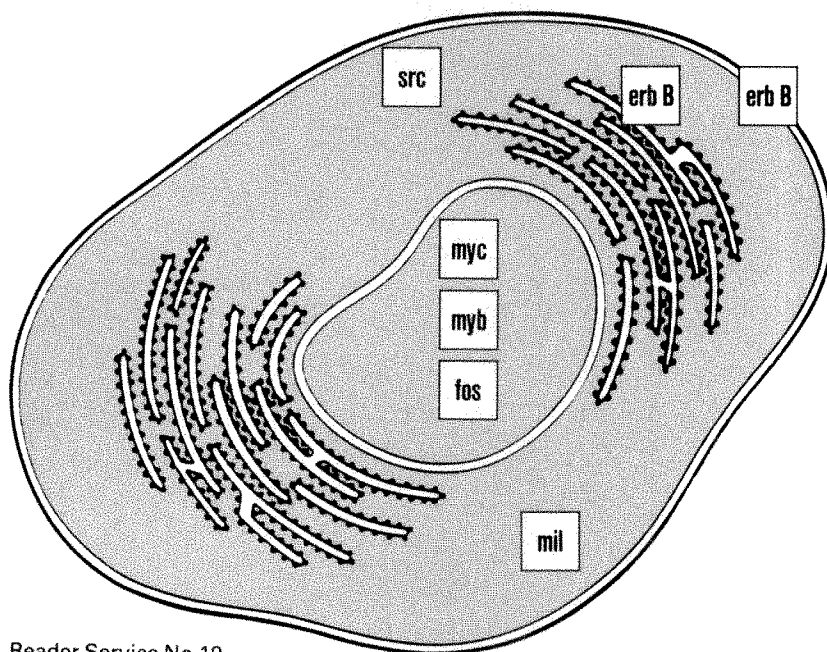
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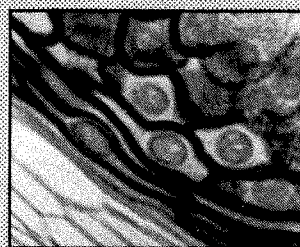
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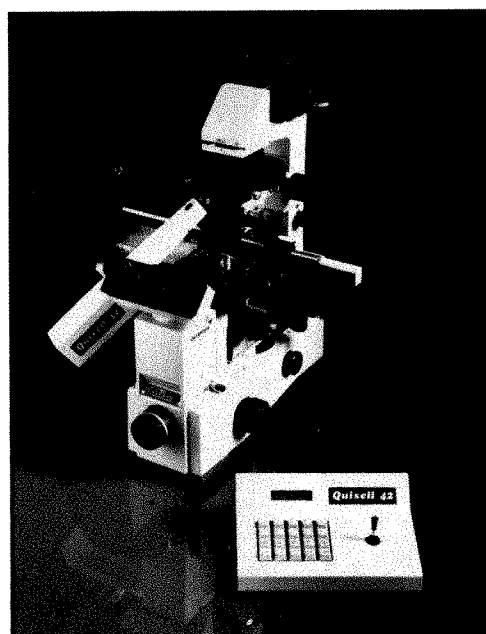
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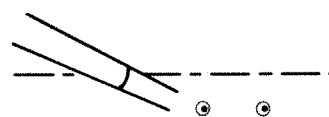
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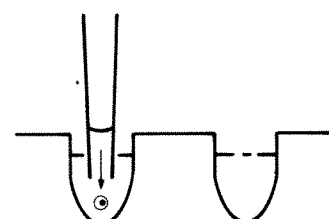
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Reader Service No.12

# Chemiluminescence lights up

I. Bronstein and P. McGrath

Chemiluminescent substrates for alkaline phosphatase and  $\beta$ -galactosidase are now available for use in immunoassays and DNA hybridization assays.

ONE of the most fascinating displays of energy release is the phenomenon of luminescence. In the broadest sense, luminescence is the conversion of energy into light. The energy required for the emission of light is generated by the oxidation of a specific substrate. Biological luminescence, or bioluminescence, occurs in organisms such as bacteria, fungi and insects<sup>1</sup>. The firefly *Photinus pyralis*, the most familiar example of bioluminescence, generates an incredibly efficient 88 per cent of its energy as luminescence. The mechanism of this reaction involves the adenylation of firefly luciferin by ATP in the presence of the enzyme firefly luciferase. Luciferin adenylate is then oxidized to a peroxide which ultimately decomposes to oxyluciferin, AMP, and  $\text{CO}_2$ , accompanied by light emission<sup>2</sup>.

The use of firefly bioluminescence in immunoassays has been demonstrated<sup>3</sup>, but the complexity and instability of the system makes this application impractical. The high background signal arising from ATP contamination further reduces the commercial potential of luciferase, because most bioluminescent assays are based on ATP detection<sup>4</sup>. Several attempts to conjugate the luciferase enzyme to antibodies have failed because of the instability of the constructs; the only successful example of labelling an anti-human antibody with bacterial luciferase is used in a rubella antibody assay<sup>5</sup>. Recently, the gene for luciferase has been cloned<sup>6</sup>, and recombinant firefly luciferase is now on the market.

## Chemiluminescent glow

Highly sensitive photomultipliers have been used to show that chemical luminescence, or chemiluminescence, is a universal property of organic substances able to undergo an oxidative reaction sufficiently exothermic to produce an emitting state. (Lightning is an example of chemiluminescence in the gases of the earth's atmosphere.) But initial expectations that chemiluminescence could be adapted readily for use in clinical diagnostics have turned to scepticism. The luminol 5-amino-2,3-dihydrophthalazine-1,4-dione, for example, when utilized as an antibody label allows only subnanomolar detection after activation with hydrogen peroxide, alkali and microperoxidase<sup>7</sup>. Peroxyoxalate chemiluminescence — generated from the reaction of oxalyl chloride, hydrogen peroxide and a

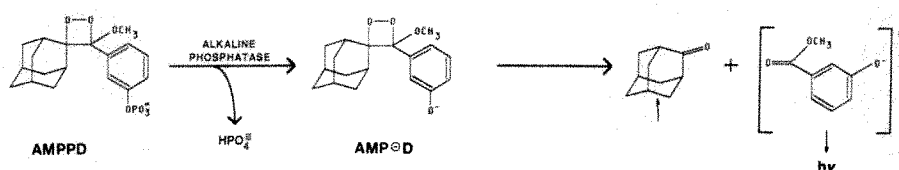


FIG. 1 Adamantyl-1,2-dioxetane phosphate (AMPPD), substrate for alkaline phosphatase, and its mechanism of chemiluminescent decomposition.

fluorescent compound such as anthracene or fluorescein<sup>8</sup> — shows very high efficiency in organic solvents, but in aqueous environments its quantum yield is considerably reduced<sup>9</sup>. The chemiluminescent signal obtained from these systems is also in the form of a short-lived (microsecond to millisecond) "flash" which requires expensive, highly sensitive photomultipliers for recording in real time.

Despite its difficulties, luminescence offers several advantages over other analytical labelling techniques: high sensitivity, low background, a wide linear range, low cost per test, and no radiation hazards. Systems which provide a chemiluminescent "glow" signal rather than a "flash" also can be detected with simple and inexpensive instruments, such as photodiode-based luminometers, and X-ray and instant photographic films.

Some luminescent labels used in bioassays can be quantified rapidly, and are relatively stable. To date, commercial chemiluminescent systems include acridium esters and enhanced luminols. (*N*-methylacridinium salts react efficiently with aqueous alkaline hydrogen peroxide to yield the excited state of *N*-methylacridone, which luminesces with a 4 per cent quantum yield<sup>10</sup>.) The basic chemiluminescent reaction mechanism is well understood, and esters which are stable for up to one year in solution have been synthesized<sup>11</sup>. The mechanism of enhanced chemiluminescent reactions is complex, and is based on the enzyme horseradish peroxidase, which catalyses the chemiluminescent oxidation of cyclic diacylhydrazines such as luminol. Luminol is oxidized by complexes formed between oxidants (hydrogen peroxide and alkali) and horseradish peroxidase, to form luminol radicals which decompose through an endoperoxide intermediate to form the excited state 3-aminophthalate dianion. When the excited state decays into its ground state, it emits light. The reaction can be improved significantly by the presence of certain phenol, naphtol, and amine "enhancers"<sup>12</sup>.

Chemiluminescent systems such as acridium esters and luminols require hydrogen peroxide, alkali, and other reagents to generate the emitting state. The addition of these chemiluminescence-stimulating reagents generally leads to a "burst" or "flash" of light, which is difficult to record by ordinary photomultiplier tube-based instruments, leading to imprecise test results.

## Flashy chemistry

Tropix has designed and synthesized a proprietary new dioxetane-based direct chemiluminescent substrate, adamantyl-1,2-dioxetane phosphate (AMPPD), for alkaline phosphatase<sup>13</sup> (Fig. 1). The AMPPD molecule is stable, emits energy at the wavelength of visible light, and has a built-in energy source (its peroxy bond). The Tropix "intelligent" molecule also

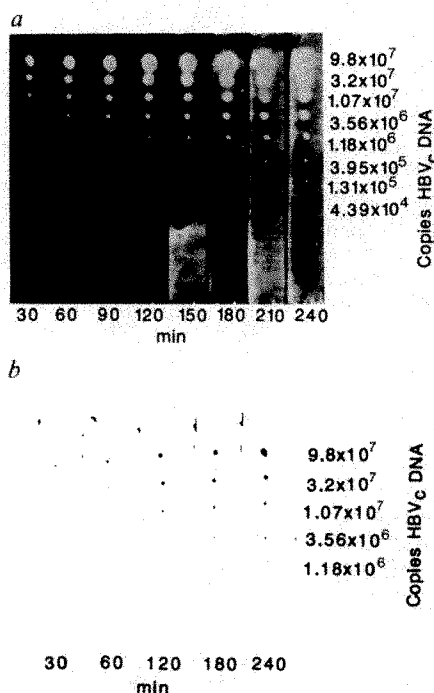


FIG. 2 a, Chemiluminescent (AMPPD) and b, colorimetric (BCIP/NBT) detection of HBV<sub>c</sub> DNA in a hybridization assay, using Polaroid Instant Black and White Type 612 film.

contains a "keyhole", specific for the alkaline phosphatase enzyme "key", which is used to unlock the stored energy of the molecule and trigger light emission. Tropix has also engineered a molecule which is activated by  $\beta$ -galactosidase.

Upon reaction with alkaline phosphatase, the AMPPD substrate spontaneously chemiluminesces. Unlike acridium esters and other common chemiluminescent systems, AMPPD is already in an oxygenated state, capable of releasing up to 100 kcal mol<sup>-1</sup> of energy. Alkaline phosphatase dephosphorylates AMPPD to form a dioxetane anion, which fragments into adamantane and the excited state of methyl *meta*-oxybenzoate anion, the light emitter<sup>14</sup>.

The activation energy for AMPPD decomposition in hydrogen peroxide is 21.5 kcal mol<sup>-1</sup>, as determined from Arrhenius plots. The measured decomposition parameters include both the thermal component, or the breakdown of the oxygen-oxygen bond, and the non-enzymatic hydrolysis of the protective group. In spite of its low activation energy, AMPPD is remarkably stable: the half-life of AMPPD in water is 142 hours at 30 °C, and in the presence of carbonate buffer at pH 12, it increases to 6,214 hours. AMPPD exhibits an indefinite shelf-life in the solid form at 4 °C. Because the nonenzymatic decomposition of AMPPD produces fragments which do not exhibit chemiluminescence, even in the presence of enzyme, there is no background signal<sup>14</sup>. Moreover, alkaline phosphatase-catalysed chemiluminescence from AMPPD is constant for prolonged periods of time. Using AMPPD, 0.001 attomole of alkaline phosphatase has been detected, on both immobilized membrane supports and in solution<sup>14</sup>. Comparable results have been obtained measuring the luminescent signal using a luminometer or Polaroid Type 612 film.

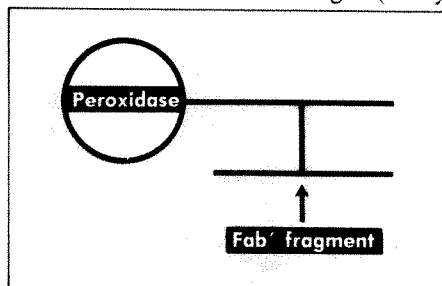
A comparison of the sensitivities of AMPPD and the chromogenic substrate 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium salt monohydrate (BCIP/NBT) in detecting alkaline phosphatase has been made in a DNA probe hybridization assay for hepatitis B virus core antigen (HBV) DNA (Fig. 2). The sensitivity of the assay was improved by more than two orders of magnitude using the AMPPD substrate for the alkaline phosphatase label. The use of AMPPD also reduced the time required to obtain a result from roughly 240 minutes to 30 minutes<sup>15</sup>.

AMPPD offers an alternative to colourimetric and fluorescent substrates for alkaline phosphatase. The substrate has been used in a broad range of assays, including direct assays for enzymes; immunoassays for proteins, haptens and microorganisms; and DNA probe assays<sup>16</sup>. AMPPD's strong signal and low back-

# Today's immunology

A fusion protein combining the best of  $\beta$ -galactosidase with Protein G, a host of human cytokines and lymphokines, and a thermally regulated microplate reader are among this week's offerings for the immunologist.

PROTOS Laboratories is now selling a line of **peroxidase-conjugated Fab' fragments** of affinity-purified antibodies (*Reader Service No. 101*). Enzyme-labelled antibodies are being used increasingly for the localization of cellular antigens in tissue sections using light or electron microscopy. To address the demand, Protos Labs has developed goat Fab' fragments linked to peroxidase which are specific for human IgG, IgA, IgM, kappa chain and lambda chain. For use in indirect staining techniques, Protos Labs offers goat Fab' anti-mouse and anti-rabbit IgG (heavy



The Protos peroxidase-Fab' fragment hybrid.

and light chains), adsorbed to insolubilized human serum. Protos Labs says its 87,000 MW Fab' conjugates penetrate tissue readily and exhibit low background staining. The Fab' conjugates cost \$80-85 (US) for 1 ml.

The **anti-phosphotyrosine fluorescein-conjugated** monoclonal antibody from Boehringer Mannheim Biochemicals is tested for its ability to stain tyrosine-phosphorylated protein in PHA-activated lymphocytes (*Reader Service No. 102*). The fluorescein conjugate supplements the company's monoclonal antibody to phosphotyrosine, which detects phos-



Boehringer Mannheim's MAB's against phosphotyrosine.

phorylated tyrosine residues produced by protein kinase activities. Boehringer Mannheim says the antibody is suited for purifying and characterizing phosphotyrosyl-growth factor receptors, transforming proteins and their substrates, and that it does not cross-react with any phosphorylated amino acids, ribose phosphate or nucleotides. The antibody can also be used for Western blot analysis, immunocytochemistry and flow cytometry.

ICN Biochemicals is offering **synthetic Lipid A** for research purposes (*Reader Service No. 103*). The company says its synthetic product has identical biological and endotoxic activities in test systems for lethal toxicity, pyrogenicity, local Shwartzman activity, Limulus Amoebocyte Lysate gelation capacity, B cell mitogenicity, induction of prostaglandin synthesis in macrophages, tumour necrotizing activity and specificity of antigenic determinants as natural Lipid A. The synthetic Lipid A is available as a lyophilized powder, with no excipient added, and costs \$85 (US) for 0.1 mg.

ground means it can be used with a variety of photosensitive detection devices, such as photomultiplier tubes, photodiodes, and photographic and X-ray films<sup>17</sup>. The utility of AMPPD in electrophoresis, blotting, sequencing, chromatography, and cytometry<sup>18</sup> is now being tested. □

Irena Bronstein and Patricia McGrath are at Tropix, Inc., 47 Wiggins Avenue, Bedford, Massachusetts 01730, USA. For more information, fill in reader service number 100.

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BRL says its **goat anti-rat IgG (H + L) antibody** and conjugates are useful for probing mouse tissues, cells or extracts with rat primary antibodies (*Reader Service No. 104*). The product is immunoadsorbed against mouse serum to remove cross-reactivity to mouse immunoglobulins. The secondary antibodies react specifically with rat primary antibodies, and are affinity-purified using rat IgG. They are available unconjugated or conjugated to horseradish peroxidase, alkaline phosphatase or fluorescein isothiocyanate.

### Cytokines and lymphokines

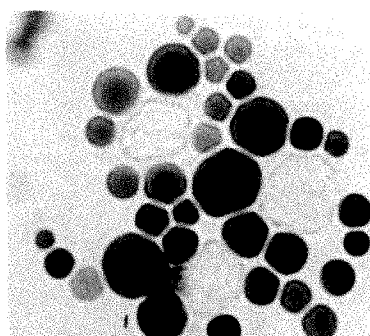
A variety of human and murine blood cell-related substances, **lymphokines and cytokines** are now available from Bachem Bioscience, Inc. (*Reader Service No. 105*). The company carries a full line of interleukins, interferons and colony-stimulating factors which are cloned, expressed, purified and tested for activity in Bachem's own molecular biology laboratories. The current list includes human interleukin-1 beta, human interleukin-2, murine interleukin-3, human interferon-gamma, and human and murine GM-CSF.

R&D Systems, Inc. sells **recombinant human cytokines** expressed, purified and assayed in the laboratories of the company from British Bio-technology's Designer Genes line of human genes (*Reader Service No. 106*). R&D Systems's range of growth factors isolated from natural sources includes bovine fibroblast growth factor alpha, bovine fibroblast growth factor beta, human and porcine tissue growth factor betas, and human and porcine platelet-derived growth factors. The company also has a line of exon-specific, anti-sense DNA probes for human interleukins, and an array of neutralizing antibodies for cytokines.

### Sorting and typing

Dynal UK Ltd has a set of **immuno-magnetic polystyrene beads** for separating and quantifying subsets of human T-lymphocyte CD4 (helper-inducer) T cells, CD8 (cytotoxic-suppressor) T cells and CD2 (Pan) T cells (*Reader Service No. 107*). The M-450 Dynabeads are uniform magnetizable polystyrene beads which bind positively to the target cells when coated with specific monoclonal antibodies. Rosettes of magnetic beads form around the T cells, allowing them to be isolated in a magnetic separation step which does not involve centrifugation. The isolated cells can be quantified or used for further studies, or the supernatant — depleted of target cells — can be analysed. Dynal says the beads do not affect cell viability, and yield preparations with better than 99 per cent purity.

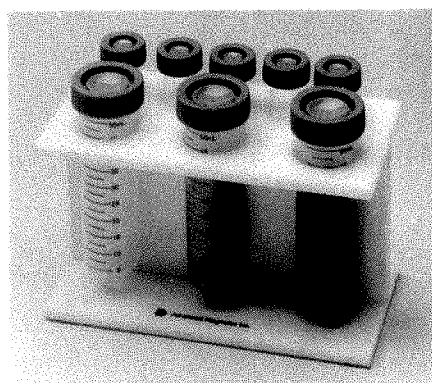
The Immunobead reagents for **human B cell labelling** from Bio-Rad consist of



Bio-Rad's Immunobead B cell labels at work.

micron-sized hydrophilic particles with covalently bound highly-purified rabbit anti-human immunoglobulin (heavy and light chains) antibodies (*Reader Service No. 108*). The Immunobead reagents simultaneously label lymphocyte surface immunoglobulins and identify any contaminating monocytes by phagocytic ingestion, says Bio-Rad. B cells are rosetted and counted using either phase or transmission microscopy, eliminating the need for fluorescent microscopy. Bio-Rad says Immunobead reagents do not react with the Fc receptors present on certain lymphocytes, so only those cells with immunoglobulin present on their surfaces are labelled. The reagent can be used simultaneously with sheep erythrocytes to rosette human B and T cells. Immunobead reagents specific for the individual human immunoglobulins IgG, IgA or IgM are available for determining subpopulations.

**Magnetic cluster of differentiation (CD) antibodies** for the isolation of specific leukocyte populations are now available



CD antibodies add to the usefulness of BioMag.

from Advanced Magnetics, Inc. (*Reader Service No. 109*). Antibodies for CD3, CD4, CD6, CD8, CD9, CD10, CD16, CD19 and CD25 are available conjugated to Advanced Magnetics's BioMag particles, allowing for the purification, depletion, enrichment and quantification of specific leukocytes. The company says its BioMag particles are non-toxic and biodegradable, and that shear forces on the leukocytes are minimized by the small 1  $\mu$ m size of the particles.

### Protein G products

The InFerGene company recently launched ProFusion  $\beta$ -Gal/G, a product which incorporates into one protein the active immunoglobulin-Fc binding sites of Streptococcal Protein G and the enzyme activity of *Escherichia coli*  $\beta$ -galactosidase (*Reader Service No. 110*). ProFusion  $\beta$ -Gal/G is a fusion protein created by recombinant means, and InFerGene says the product has a greater batch-to-batch consistency and a higher specific activity than similar conjugates made by chemical coupling reactions. InFerGene is now selling ProFusion  $\beta$ -Gal/G directly, and through its American Enzyme Corporation subsidiary.

The GammaBind G HPLC columns from Genex are affinity columns packed with a pH-resistant, polymer-coated silica solid support bound to recombinant protein G (*Reader Service No. 111*). Genex has engineered the protein G in the GammaBind G columns to increase its

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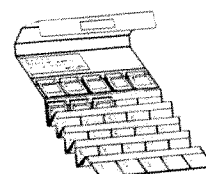
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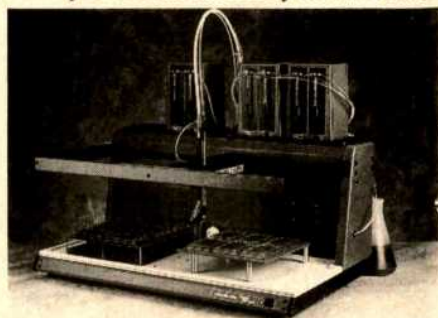
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natural resistance to proteolytic degradation. The company says the recombinant product binds specifically and only to all subtypes of IgG antibodies, with no cross reactivity with other serum proteins or IgM, IgE or IgA antibodies. The GammaBind G columns can be used for purifying or quantifying IgG antibodies in serum, ascites fluid, and hybridoma cell culture supernatants; measuring IgG levels for quality control and process monitoring; concentrating and purifying IgG monoclonal antibodies from tissue culture media; removing IgG from serum samples; and purifying mitogen-free antibodies for lymphocyte studies. The GammaBind G columns come in analytical and semi-preparative sizes, and column adaptors are available to fit them to most HPLC systems.

### Harvesting and diluting

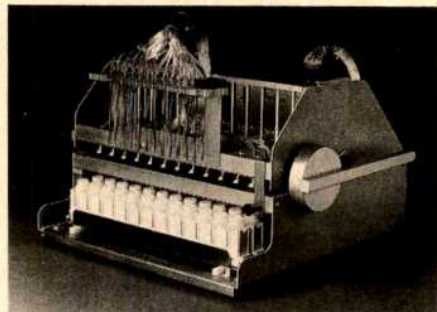
Hamilton Company has introduced a new multi-probe head accessory for its Micro-



Hamilton's head for diluting/dispersing. lab 2200 **automated liquid handling station** (Reader Service No. 112). The multi-probe head utilizes up to three Microlab 941 dual-channel diluters/dispensers as well as the two internal diluter channels in the Microlab 2200. It allows spacing between probes to be varied from 9 mm up to 21 mm. Up to eight independent reagent transfers can be performed simultaneously, reducing sample preparation time, says Hamilton. The multi-probe

head can be used to add reagent to tubes or microplates by aspirating and dispensing liquid through probe needles; to add reagent from large reservoirs by pumping through the syringes and tubing; and to perform serial dilutions in microtitre plates.

Cambridge Technology, Inc. has a **sample harvester** which it says can punch, harvest and deposit samples directly into



Model 2000 PHD sample harvester from Cambridge Technology.

any type of scintillation vials (Reader Service No. 113). Cambridge Technology's model 2000 PHD sample harvester has a large-diameter collection area and the ability to handle large samples — a plus for receptor binding assays. The \$4,800 (US) model 2000 harvests 24 samples at a time onto 25 mm collection sites, and inserts them into standard test tubes, gamma counter tubes, minivials or 20-ml scintillation vials, depending on which sample tray is used. Several grades of pre-cut glass fibre filter strips are available to go with the sample harvester.

### Plate readers

Molecular Devices has a **thermally regulated microplate reader** which allows researchers to perform valid kinetic anal-



The THERMOmax from Molecular Devices.

yses at elevated temperatures (Reader Service No. 114). The THERMOmax from Molecular Devices controls temperature from 4 °C above ambient temperature to 42 °C, with a resolution of 0.1 °C and a temperature variation across the microplate of less than 0.5 °C, says the company. The microplate can be kept stationary for turbidimetric assays, such as clot formation assays for the detection of endotoxin, or clot dissolution assays for the detection of TPA. For assays where mixing is necessary, such as bacterial growth studies, the instrument's Automix function minimizes the settling of cells. The SOFTmax 2.0 IBM-compatible soft-

ware for the THERMOmax performs nonlinear kinetic analyses and computes time to reach  $V_{max}$ . The software also includes five standard curve algorithms and a set of displays and reports.

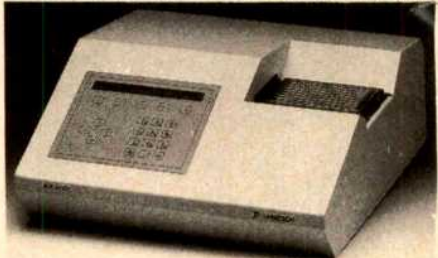
Millipore's new CytoFluor **fluorescent measurement system** is a computer-controlled scanning device for multiwell plates which can be used to quantify both



Less than one-minute measurements with Millipore's CytoFluor fluorescent measurement system.

soluble and cell-associated fluorescent signal, for applications in cytotoxicity testing, phenotyping, cell receptor labelling, and fluorescence-based enzyme assays (Reader Service No. 115). Millipore says the instrument reads fluorescent signal in less than one minute through standard tissue culture plastic or through membrane-based tissue culture devices. The CytoFluor system can accommodate 6-, 12-, 24- or 96-well plates, and allows multiple wavelength scanning. All operations are software-driven, and data is reported as either an EXCEL spreadsheet or a comma separated values (CSV) file for fast data analysis.

Dynatech says its MR 5000 **kinetic microplate reader** is capable of reading an entire microplate in 1.7 seconds (Reader Service No. 116). The MR 5000 has a built-



Dynatech's microplate reader for kinetic analyses.

in microprocessor capable of storing up to 50 different user-defined test protocols, and the data from 20 microplates. The integral software allows for data analysis on any of the stored data, as well as on a newly read plate. □

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## INSTITUTE OF VIROLOGY AND ENVIRONMENTAL MICROBIOLOGY (OXFORD)

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## MICROBIAL GENETICS, MOLECULAR BIOLOGY AND MICROBIAL PLANT ECOLOGY

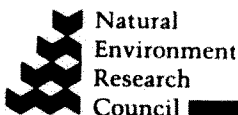
With the expansion of the remit of the NERC Institute of Virology to include environmental microbiology, and the change of the name to the INSTITUTE OF VIROLOGY AND ENVIRONMENTAL MICROBIOLOGY, a new program of research in environmental microbiology has been launched.

Three new staff appointments have been created, two at the post-doctoral, (HSO), level and one at the post-graduate, (SO), level. We are looking for highly motivated individuals, with experience in prokaryotic genetics and molecular biology who would be interested in developing a research career in this important area. Knowledge of microbial ecology would be an asset. The initial research objectives of the group include the quantification and study of the fate, transfer and persistence of genetically engineered bacteria and their DNA in the environment following release onto plant surfaces, thereby leading to an understanding of the potential risk and environmental impact.

Salary will be in the range of £8,574-£10,994 for Scientific Officer appointments and £10,026-£13,460 for Higher Scientific Officer appointments. Starting salary depends on experience and qualifications. Staff of the Council are not Civil Servants but their pay and conditions are similar to those on the Civil Service. There is a non-contributory pension scheme.

Application forms are available from the **Administration Officer, Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, OX1 3SR. Telephone: Oxford (0865) 512361.** Informal enquiries may be made to Dr Mark J Bailey, at the above address, for further details of the posts available.

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(NW3582)A

## UNIVERSITY OF BERNE SWITZERLAND YEAST MOLECULAR GENETICS GROUP LEADER POSITION POSTDOCTORAL POSITION

Applications are invited for the position of leader of a small research group from persons with postdoctoral experience. It is expected that the appointee develops his independent research program in the molecular analysis of genetic recombination in yeasts. Collaboration with the existing group is welcomed. Teaching obligations are minimal, but allow the recruitment of Ph D students. Appointment to this university position is for three years initially, with possible extensions (Habilitation-stelle). The position opens on the beginning of 1990.

Applications are invited for a postdoctoral position available this summer for the study of genetic recombination in fission yeast. Persons with experience in DNA research and protein-DNA interactions are preferred. The position is funded by the Swiss National Science Foundation. Appointment will be for two years initially.

The current projects of the group are aimed at the study of DNA recombination mechanisms in fission yeast. The approaches include formal genetics, cloning and sequencing of genes, study of gene expression, in vitro mutagenesis and yeast transformation, identification of recombination intermediates and enzymes.

For further information and applications (curriculum vitae, names of references), please contact **Prof. J. Kohli, Institute of General Microbiology, University of Berne, Baltzer-Strasse 4, CH-3012 Bern, Switzerland** (Telephone: 031 65 46 54). (W6039)A





Applications are invited for the following posts 4 of which are under the New Academic Appointments Scheme.

## DEPARTMENT OF ANATOMY LECTURER (NAAS)

Qualified in any relevant field.  
(Ref 89/34)

## SCHOOL OF PURE AND APPLIED BIOLOGY LECTURER (NAAS)

Applied Insect Taxonomy and Tropical Pest Management.  
(Ref. 89/35)

## SCHOOL OF CHEMISTRY AND APPLIED CHEMISTRY LECTURER PHYSICAL (NAAS)

Surface science or catalysis research interests preferred.  
(Ref. 89/37)

### LECTURER: ORGANIC

Medicinal Chemistry research interest preferred. (Ref. 89/38)

### LECTURER ANALYTICAL (NAAS)

Sensing processes and transducer technology interest. (Ref. 89/39)

**Salary: Lecturer £9260 – £19310 per annum (under review).**

For further particulars and application form please write to  
**University of Wales College of Cardiff, PO Box 431,  
Cardiff CF1 1TA quoting appropriate reference number.**

Closing Date: 5 May 1989

(8990)A

## UNIVERSITY OF READING

### School of Animal & Microbial Sciences

(Department of Pure & Applied Zoology)

Applications are invited for a Lectureship from 1 September 1989 (under the New Academic Appointments Scheme) in the Department of Pure & Applied Zoology with special responsibility to teach and research in the area of insect physiology and molecular biology of insects, particularly in relation to crop protection and pest control. Consideration would also be given to applications from persons working in appropriate fields but with non-insect pests. This post would further strengthen both the Department's and School's considerable activities in insect physiology and pest control. Initial salary up to £12760 p.a. on the Lecturer Grade A scale (£9260-£14500 p.a. under review) plus USS benefits. Further particulars and application forms (2 copies) are available from the

**Personnel Office, University of Reading, Whiteknights, PO Box 217, Reading, RG6 2AH.** Please quote Ref. AC. 8922. Informal enquiries to the Head of Department, Dr. A.R. Jones.

Closing date 12 May 1989.

(8962)A

## FACULTY POSITION DEPARTMENT OF BIOCHEMISTRY

The Department of Biochemistry is looking for candidates for a full-time faculty position available from July 1st, 1989.

The department is responsible for two undergraduate programs (biochemistry and microbiology) and two graduate programs.

The department has two research groups: an oral ecology group (GREB) and a group on molecular genetics (CRGM). Research activities of the GREB include fundamental aspects of microbiology, biochemistry, immunology and molecular biology related to the pathogenic mechanisms of dental caries, periodontal diseases and oral candidosis. Members of the CRGM group have a common interest in the regulation of gene expression and gene evolution while studying a variety of biological systems.

Applicants must hold a Ph.D. degree in microbiology or biochemistry and have at least one year of postdoctoral experience. The candidates must have a proven skill in written and oral French or be willing to acquire it within a year. The successful candidate:

- i) should preferably have an experience in one of the following fields: protein engineering, host-parasite interaction, virology, immunogenetic or membrane biology,
- ii) will be part of either one (or both) of the departmental group, and
- iii) will participate in the regular teaching of the department.

Applicants should submit their curriculum vitae, a statement on how they will integrate themselves in the departmental research groups and arrange to have three letters of recommendation sent to: **Denis Mayrand, Chairman, Département de biochimie, Faculté des sciences et de génie, Université Laval, Québec, Canada, G1K 7P4, (Tél: (418) 656-3016, FAX: (418) 656-5902).**

Deadline for receipt of applications is June 15, 1989. In accordance with Canadian Immigration requirements, this advertisement is particularly directed to Canadian citizens and permanent residents. *An equal opportunity employer (M/F).* (NW3589)A

## POSTDOCTORAL POSITIONS

**Public Health Service,  
National Institutes of  
Health, Research Triangle  
Park, North Carolina**

Postdoctoral scientists are needed in the Laboratory of Molecular Genetics of the National Institute of Environmental Health Sciences to investigate mechanisms of DNA replication, recombination and repair in the yeast *Saccharomyces cerevisiae*. Ph.D. or equivalent required in biochemistry, molecular biology, biology, microbiology, genetics or a related discipline. Salary range \$20,000 to \$28,000 for entry-level candidates, commensurate with background.

For additional information, contact Dr. Akio Sugino at 919-541-7732. To apply, submit curriculum vitae, bibliography, statement of future research interests and three letters of reference by May 30, 1989 to:

**Bruce Wiggins (HNV1);  
NIEHS Personnel Office;  
P.O. Box 12233; Research  
Triangle Park, NC 27709.**

**An Equal Opportunity Employer**

(NW3586)A

## MEDICAL RESEARCH COUNCIL LABORATORIES

THE GAMBIA

**Molecular biologist, MRC  
Laboratories, Fajara**

Applications are invited from suitably qualified scientists, preferably at the post-doctoral level, for a post as a molecular biologist at the MRC Laboratories, The Gambia. The successful applicant will join a team studying the infectious diseases such as malaria, pneumonia, measles, and HIV infection which are prevalent in tropical developing countries. He or she will be expected to develop a molecular biology laboratory in which molecular biological techniques can be used in diagnosis and in study of the epidemiology of infection diseases. Previous experience with the PCR technique would be an advantage.

The duration of the contract that will be offered is flexible; this might include a period in an established European molecular biology laboratory. Salary, which will be on the MRC International scale, will be determined by previous qualifications and experience.

Applications should be submitted to **TREVOR FLINDALL**, MRC Head Office, 20 Park Crescent, London W1N 4AL UK from whom further details may be obtained. The closing date for this application is 12 May 1989.

(8960)A

**Sandoz is a leading pharmaceutical company with an active research commitment in the field of cardiovascular medicine. We are looking for a**

# CELL BIOLOGIST

to join a new group within our cardiovascular department. As part of an interactive team the work will involve a basic research effort using cellular and molecular biology to address the pathology and treatment of cardiovascular disease.

The successful candidate will be in charge of his/her own laboratory and will be responsible for the establishment, characterization and maintenance of primary cultures and cell lines. Experience with endothelial cells/hepatocytes, immunohistochemical and in situ hybridization techniques would be an advantage.

Candidates who have a PhD or MD with research or industrial experience are invited to send a curriculum vitae including a list of publications, references and a statement of research interests to:

**Sandoz Ltd., Personnel Department, Ref. 9103,  
attention Dr. R. Racine (061 24 24 66)  
CH-4002 Basle, Switzerland**



(W6041)A

## MAMMALIAN CELL CULTURE

### SENIOR RESEARCH SCIENTIST

Celltech is one of Europe's foremost biotechnology companies. We are among the leaders in the research and manufacture of protein-based drug products, and with our portfolio of successful candidate therapies, we are establishing a fully integrated biopharmaceutical capability.

We are now looking for an experienced life scientist to join our fermentation physiology team. Working on the development of mammalian cell lines and processes for the manufacture of therapeutic proteins, you will investigate factors affecting productivity and product quality, screen and select candidate cell-lines, and develop appropriate media and fermentation regimes. This will involve close collaboration with Celltech's scientists in other areas, such as molecular biology, fermentation scale-up and protein purification.

Aged between 25 and 35 and with a PhD in a biological science, your most likely current R & D role will be in academia or industry; your research experience up to and, ideally, beyond doctoral level should include knowledge of tissue/cell culture. You will have the creativity and self-confidence to work as part of a scientific team, with the potential to lead it; good communication skills, written and verbal, are essential.

A highly competitive salary will be supported by a comprehensive benefits package which includes a pension scheme, share save scheme and relocation assistance if appropriate.

Please write, with full details of your qualifications and experience to date, to Mrs Jane Smith, BSc C BIOL MI BIOL, at Celltech Limited, 216 Bath Road, Slough, Berkshire SL1 4EN. Please quote Ref: 271.



(9005)A

## DEPARTMENT OF PSYCHIATRY UNIVERSITY OF TORONTO

### Applications are invited for the position of ASSISTANT PROFESSOR

in the area of Molecular Neurogenetics as applied to psychiatric disorders. This position requires a Ph.D. or other doctoral degree and significant post-doctoral experience in molecular genetics, molecular neurobiology or an equivalent field. The successful candidate will be expected to work collaboratively with a multidisciplinary neuroscience/neuropsychiatric research group comprised of basic and clinical researchers investigating the neurobiology and neurogenetics of psychiatric disorders. The position requires demonstrated ability to conduct independent research within a collaborative group and to compete for extramural funding.

The position is tenure track equivalent supported initially by the I'Anson fund. Salary is commensurate with rank and experience. The position also includes a one time start up research grant.

The University encourages both men and women to apply for positions. In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada.

Candidates should submit a letter of application, a curriculum vitae and the names of three referees from whom letters of recommendation may be solicited. Please submit responses to: **Dr. Jerry I. Warsh, Director of Research, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario M5T 1R8.**

The successful candidate would be expected to take up the appointment prior to September 1, 1989.

**Closing date for applications is May 19, 1989. (NW3559)A**



## GLAXO INSTITUTE FOR MOLECULAR BIOLOGY S.A.

The Geneva biotechnology research unit of the largest U.K. pharmaceutical group has a vacancy for a

# Biochemist

to investigate  
**membrane receptors**  
**signal transduction mechanisms**

The Glaxo Institute for Molecular Biology applies a molecular approach to the discovery of effective new medicines. Projects include inflammatory diseases, viral therapeutics, neurobiochemistry and the regulation of cell proliferation and differentiation.

The Institute has a staff of over 120 people, grouped in small teams of specialists from several domains of modern biology. We are part of the Glaxo Group, which has an outstanding reputation for the quality of its research and products. We offer an excellent salary and benefits package, an interactive research environment, and opportunities for career development in one of the most attractive parts of Europe.

The ideal candidate will have several years of postdoctoral research experience and a proven track record in the field of membrane receptors or signal transduction mechanisms. Experience with animal cell cultures would be an advantage.

If you are interested in this position, please send your curriculum vitae with a list of publications and the names of three references to: Rita Gloor, Personnel Manager, GLAXO IMB SA, 46, route des Acacias, 1211 Geneva 24, Switzerland.

(W6040)A

### UNIVERSITY OF ABERDEEN UNIVERSITY OF ESSEX PROSAMO

#### POSTDOCTORAL AND GRADUATE RESEARCH ASSISTANTS AND TECHNICIANS IN MICROBIOLOGY, IMMUNOLOGY and ENVIRONMENTAL BIOTECHNOLOGY

PROSAMO is a major new interdisciplinary DTU/industry/AFRC funded three year project to develop methods that will provide the basis for assessment of the risks arising from the release of genetically manipulated plants and micro-organisms into the environment. The microbial programme will require the co-ordinated activities of a team of scientists at both Aberdeen and Essex Universities.

Applications are invited for the following posts from those with experience in microbiology, immunology, biochemistry, molecular biology or soil science.

At the *University of Aberdeen*: to work on the cloning of the *lux* gene system in soil bacteria, the construction of luminescent bacteria and the development of methods to extract and trace very small numbers of such bacteria added to laboratory microcosms and field samples.

3 postdoctoral (Ref LW/016) and 1 graduate research assistant (Ref LW/017), one of the postdoctoral positions to have a combined role in both centres.

At the *University of Essex*: to work on the development of polyclonal and monoclonal antibodies to use with fluorescent probes to identify genetically manipulated strains of bacteria in soil, detecting bacteria by means of flow cytometry and fluorescence activated cell sorting.

1 senior research officer (PDRA: Scale IA) (Ref R/864) and 1 Grade 5 technician (Ref N/865).

Salaries: PDRA, Scale IA (£9865-£12760); PGRA, Scale IB (£8675-£11680); Technician, Grade 5 (£8088-£9549). Appointments are renewable annually for up to three years.

Applications (3 copies) with full CV and the names of two referees should be sent to **The Personnel Office, University of Aberdeen, Regent Walk, Aberdeen, AB1 1FX** (for references LW/016 and LW/017) or to **The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ** (for references R/864 and N/865). Further details for the appropriate posts may be obtained from either of the above addresses. The closing date for applications is 5 May 1989. (8965)A

### UNIVERSITY OF READING

#### DEPARTMENT OF BIOCHEMISTRY AND PHYSIOLOGY

#### Postdoctoral Research Fellow and Graduate Research Officer

Applications are invited for the above posts in a new well equipped group to be set up in Reading actively to investigate at a biochemical level the role of lipoproteins in coronary artery disease. The projects will entail mainly tissue culture and studies of lipoprotein modification, for which full training will be given. The Fellowship is for one year in the first instance and is supported by the Wellcome Trust (salary up to £12760 p.a. under review). The Officership is for 2 years 3 months and is supported by the MRC (initial salary £8765 p.a. under review). Both posts are available immediately but starting dates are negotiable. Informal enquiries may be made to Dr. David S. Leake on 01-873-2315 or, after 1 May, (0734) 875123.

Apply for Application Form to **Personnel Officer, University of Reading, Whiteknights, P.O. Box 217, Reading RG6 2AH**. telephone (0734) 318754. Please quote Ref. R8922. (8961)A

### INSTITUTE OF CHILD HEALTH UNIVERSITY OF LONDON

#### Paediatric Cardiology MLSO Grade 3

Salary £15,711 to £17,515 inclusive

Skills essential:

- 1) Preparation of material for light and electromicroscopic examination, including Immunohistochemical techniques.
- 2) Experience with cell culture highly desirable.

The post includes responsibility for training and supervising laboratory staff.

To work in a high calibre research group in the Department of Paediatric Cardiology at the Institute of Child Health.

Further particulars available from the **Personnel Assistant, 30 Guilford Street, London WC1N 1EH** (tel 01-242 9789 ext 2650) to whom completed applications (3 copies) including the names of two referees, should be returned by 14 May 1989. Ref. MLSO3/PC/4/89. (8972)A



## THE GERTRUD – HAGMANN – STIFTUNG für Malignom – Forschung

has been established in Basle, Switzerland for the support of scientists performing basic or clinical research in oncology. Conditions for applicants are as follows:

1. Applicants must have obtained the doctoral degree either in Science or in Medicine.
2. They must have performed basic or clinical studies in biology or medicine at least two years prior to the application.
3. A funded scientist has to carry out his studies either in Austria, the Federal Republic of Germany or Switzerland.
4. He must be free of routine service or teaching obligations.

The stipend is sFr. 50,000.– per annum and shall provide the living expenses for the successful applicants.

Payments will be three-monthly in advance for two years. Reapplication is possible.

The Foundation will be gratified to receive applications of qualified female candidates.

Applications should be submitted within two months of this announcement to the **President Rainer Hagmann, M.D., Gertrud-Hagmann-Stiftung für Malignomforschung, Hauptstr. 14, CH 4514 Lommiswil, Switzerland**, and include the name and address of the applicant, a curriculum vitae, a complete list of publications including three copies of the most important papers, further a detailed description of the research project with references to previous works, the address of the institution where the investigation should be carried out with written consent of its director, specifying the time, when research should be started.

The council of the Foundation, with Prof. K. Brunner, M.D., Director of Oncology at the University of Berne, and Prof. G. Hartmann, Director at the Institute for Biochemistry at the University of München, serving as members, will notify the applicants of its decision within three months after the closing date of applications. (W6049)A



## Cancer Research Campaign

Fighting cancer on all fronts

Beatson Institute for Cancer Research

### POST-DOCTORAL SCIENTIST

A post-doctoral scientist is required to join a group working on the role of cell-cell interactions in the control of cell growth and differentiation, carcinogenesis and tumour suppression. The position offers an opportunity to develop an independent research project within an established epithelial cell biology and carcinogenesis programme. Candidates should be interested in the application of molecular and genetic techniques to problems of cell biology and cancer. There are good facilities for clinical collaboration when appropriate. Experience in cellular and/or molecular biology would be an advantage but training is available for applicants with proven ability in other fields. The appointment, for three years in the first instance, is funded by the Cancer Research Campaign as part of an ongoing research programme. It will be on the MRC Grade II scale (currently £11,070 to £14,500) with USS superannuation.

Applications, including a C.V. and the names of two referees, should be sent as soon as possible to **Dr John D. Pitts, Beatson Institute for Cancer Research, Garscube Estate, Bearsden, Glasgow, G61 1BD** (Tel: 041 942 9361). (8985)A

### JUNIOR FACULTY POSITION

available in the pharmacology and biochemistry of antineoplastic agents targeted against specific human cancer types. Studies will involve the modulation of anticancer agents with thymidylate synthase as the primary target. The focus will be on metabolism of reduced folates, modulation of reduced folate pools, and optimization of drug scheduling for therapeutic gain. Research will also identify factors that influence the regulation and turnover of thymidylate synthase in elucidating the importance of the enzyme as a therapeutic target. Candidates should have the Ph.D. degree with a minimum of 3 years postdoctoral experience. A strong background in Biochemistry is advantageous. Send curriculum vitae and names of 3 references to:

**Dr. J. A. Houghton,  
Laboratories for Developmental Therapeutics,  
Department of Pharmacology,  
St. Jude Children's Research Hospital,  
332 North Lauderdale, Memphis, TN 38101.**

Equal Opportunity/Affirmative Action Employer. (NW3556)A

## Molecular Biologists

### Two vacancies for experienced Research Scientists

Fisons is one of the most prestigious British pharmaceutical companies with an excellent reputation and clear commitment to new drug discovery. The company has made a substantial investment in immunological, cardiovascular and respiratory research and now wishes to establish a molecular biology unit within its Research laboratories in Loughborough.

Molecular biologists are required initially to support our immunosuppressive and cytokine programmes, but in the longer term to contribute to our receptor based approaches to new treatments for cardiovascular and respiratory diseases.

Applicants will have a PhD with up to three years relevant post doctoral experience. In addition, they should have a proven record of original research in molecular biology. A second position requires applicants who have graduated in biochemistry and have had experience in research using molecular biological techniques.

Successful applicants for both positions will need to be innovative and have the ability to communicate and implement ideas and to interact successfully with their colleagues as these are key positions within our expanding department of Biochemistry.

We offer excellent conditions of employment which include: profit sharing bonus, flexitime, a competitive pension scheme, 25 days annual holiday plus generous relocation assistance where applicable.

Please send your c.v. or write for an application form to:  
**Mrs Jane Thompson—Personnel Manager  
Fisons plc—Pharmaceutical Division  
Research and Development Laboratories  
Bakewell Road  
Loughborough  
Leics  
LE11 0RH**

**FISONS**

Pharmaceuticals

(8968)A

Please quote reference number rd183/N on all correspondence

## PROFESSOR OF ASTROPHYSICS WITH COSMOLOGY\*) AT STOCKHOLM OBSERVATORY

The University of Stockholm hereby announces a position as professor of astrophysics with cosmology at Stockholm Observatory open for application. The successful candidate will be expected to be actively engaged in research, to participate in teaching (in Swedish or English) including supervision of graduate students, and to take part in local, national, and international administration duties of the Observatory.

\*)The area of responsibility for the position covers the whole of astrophysics including cosmology. The candidate is required to have a scientific background within *either one* of these areas. The specialization can be observational or theoretical.

Application: The application should be made to "The Swedish Government" and the mailing address is:

**Rektorsämbetet  
Stockholm University  
S-106 91 STOCKHOLM  
Sweden**

The application should be accompanied by:

1. Detailed curriculum vitae and bibliography
2. Short written account of scientific work and teaching experience
3. Copies of records or documents that the applicant wishes to submit to verify his qualifications
4. Four reprints or preprints of each of the applicant's scientific publications numbered according to the publication list

Closing date: The formal application and documents under item 1 above must be received by the University not later than **May 8, 1989**. (It is advisable to submit the formal application by telex to 8105199 UNIVERS or telefax (0)8-159522.)

Documents under items 2-4 above must be received by the University not later than **May 29, 1989**. (W6038)A

# SENIOR POSTS IN RESEARCH ADMINISTRATION

The Medical Research Council wishes to appoint a small number of scientists to join its team of Scientific Administration Officers at the MRC Head Office in London.

Scientific Administration Officers act as one of the main channels of communication between those in universities, hospitals and research institutes whose research is supported by the MRC or who are seeking support, and the Council and its decision-making Boards and Committees. Each is assigned to take responsibility for the administration of research support in a specific scientific field.

Minimum qualifications are a good honours degree in a relevant science subject together with at least four year's relevant research experience. The successful candidates are likely to have had some proven post-doctoral research experience in an academic or commercial environment. Candidates will need to demonstrate a broad working knowledge of science and medicine, and have the analytical, communication and representational skills necessary to handle, after training, the complex administrative aspects of research management.

This is an unusual and challenging opportunity to move from research into strategic research administration. Appointments will be to the Senior Scientific Officer grade, which has a current salary of £14693 to £18782 (including London Weighting) plus a 4.5 per cent supplement for those joining the MRC Pension Scheme. Additional performance payments are available for the best staff. Conditions include pleasant office surroundings near Regents Park and flexible working hours.

**MRC**  
Medical Research Council

For application forms and further particulars apply by letter or telephone to **HQ Staffing Group, Medical Research Council, 20 Park Crescent, London W1N 4AL**. Tel 01-636 5422, Ext 447. Closing date: 5 May 1989. (8973)A

## ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London) Department of Pharmacology CNS ELECTROPHYSIOLOGISTS

To study voltage and transmitter gated conductances of thalamic neurones, with particular emphasis to the thalamo-cortical projection cells of the lateral geniculate nucleus and the gabaergic cells of the perigeniculate nucleus.

Two post-doctoral positions are currently available for 17 and 36 months. They are supported by the Wellcome Trust with salary up to £11,680 (RIA scale) plus £1,650 London allowance per annum. Previous experience with electrophysiological techniques advantageous, but not essential.

Applications, with curriculum vitae and the names of 2 referees, should be sent to **Dr V Crunelli, Department of Pharmacology, St George's Medical School, Cranmer Terrace, London SW17 0RE, from whom further particulars can be obtained (tel. 01-672 9944 ext 55625). Please quote reference 48/89.** (8984)A

## CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL (University of London) DEPARTMENT OF MEDICAL ONCOLOGY and CANCER RESEARCH CAMPAIGN LABORATORIES RESEARCH ASSISTANT

Applications are invited from post-doctoral biochemists and pharmacologists for a post supported by the Cancer Research Campaign. This post involves the development and pharmacology of a variety of potential new drugs in the treatment of cancer. Experience of pharmacological techniques and in particular HPLC is relevant. Salary within range £9,865-£15,720 p.a. plus £1,650 p.a. London Allowance, depending on age, qualifications and experience.

Applications in writing with full curriculum vitae together with the names and addresses of two referees to **The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP**, to be submitted by 1st May 1989. (Quote Ref: 89/30) (8945)A

## UNIVERSITY OF ABERDEEN DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY EMBRYOLOGIST

The Department of Obstetrics and Gynaecology, University of Aberdeen is setting up a Self-funding IVF Unit. This will be run within the University Department, but will have close links with an established NHS regional referral clinic. Facilities have been developed within a successful and productive research programme. Current projects include embryo diagnosis (cytogenetics and DNA probes) and ovarian control of gonadotrophin secretion. Our requirement is for an experienced embryologist with the ability to initiate an active clinical programme and at the same time take full advantage of the research opportunities. The appointment would be for one year in the first instance and salary will be within the range £9,865-£16,345 on the Research and Analogous Scales.

Informal enquiries to Professor Allan Templeton, Department of Obstetrics and Gynaecology, Maternity Hospital, Foresterhill, Aberdeen (tel 0224 681818 ext 52456).

Applications forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** (tel 0224 273500) to whom applications (2 copies) should be returned by 12 May 1989 quoting reference number LW/019. (8956)A

## THE UNIVERSITY OF THE SOUTH PACIFIC

Alafua Campus, Western Samoa

## FELLOW IN ATOLL AGRICULTURE (Post 89/19)

Applications are invited for the post of Fellow in the Institute for Research, Extension and Training in Agriculture. The Institute is centred together with the School of Agriculture on the University's campus at Alafua in Western Samoa. The Fellow in Atoll Agriculture will be based upon a sub-station located in Tarawa, Kiribati, and will be responsible to the Director of IRETA for the continuation of ongoing research programmes into systems of farming to sustain yields of food crops, particularly vegetables, in an atoll environment. Applicants should possess a good first degree in agriculture, horticulture or applied science with preferably a higher degree in Agronomy or soil science and experience of tropical agriculture preferably in an atoll environment. Appointment, pending final confirmation of funds approved in principle, will be for a three year contract period with a possibility of extension for a further two-year period.

Salary will be in accordance with qualification and experience in the following scales: Junior Lecturer A\$14,154-15,882; Lecturer II A\$16,515-18,479; Lecturer I A\$19,132-A\$26,001; Senior Lecturer A\$26,833-31,354. Funding allows only citizens of the region and the European Community to be appointed. The University also provides gratuity amounting to 15% of basic salary; appointment allowance; partly furnished accommodation at a rental of 12.5% of salary; and a contribution of 10% of basic salary towards an approved superannuation scheme.

Further information may be obtained from the Secretary (Alafua Campus) (Telephone 21671; Telex 251 SX; Fax (685) 22933).

Candidates should send **THREE COPIES** of their curriculum vitae with full personal particulars, names and addresses of three referees and date of availability. In order to expedite the appointment procedure applicants are advised to ask their referees to send confidential reports direct to the University without waiting to be contacted. Applications should be sent to the Secretary, School of Agriculture, University of the South Pacific, Alafua Campus, Private Mail Bag, Apia, Western Samoa to reach him no later than **28 April 1989**. Applicants resident in the UK should also send a copy to **Appointments (36230), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.** (W6046)A

MOLECULAR DESIGN OF NEW CROP-PROTECTION AGENTS

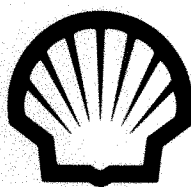
# Biophysical/ Physical-Organic Chemist

At Shell Research's Sittingbourne Research Centre, Molecular Design brings together many skills in a large multi-disciplinary team supported by extramural research. We have a vacancy in this team for a scientist with skills in biophysical or physical-organic chemistry.

A clear understanding is needed of the properties required in effect-chemicals and of how these may be realised in a molecular structure. We are looking for scientists experienced in characterising and quantifying enzyme functions and interaction with substrates and inhibitors, using spectroscopic and other physical methods. We also invite applications from people experienced in investigating transport and related physical processes which determine a compound's properties within an organism and its delivery to a target. We seek candidates who have academic or industrial postdoctoral experience in an appropriate field, good communication skills and a willingness to work across traditional scientific boundaries.

Our Research Centre is a modern, well-equipped laboratory on a 500 acre rural site in Kent, offering a wide range of intellectual challenges and opportunities for career development. We offer attractive salaries, good sports and social facilities and a staff restaurant.

If you are interested in the above position, please write or telephone for an application form, quoting reference number SRC5/89 (Biophysical Chemist) to: Mrs Diane King, Personnel Division, Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG. Tel: (0795) 412290. Closing date for receipt of applications 27th April 1989.



Royal Dutch/Shell Group

CAREERS OF CONSEQUENCE

(9006)A

**nature**

the widest international selection of jobs  
in science — EVERY WEEK

**STRANGWAYS RESEARCH LABORATORY**  
Worts Causeway, Cambridge CB1 4RN

### **Post-doctoral Biochemist**

required to take part in a broad programme of research on medical aspects of proteolytic enzymes and their inhibitors. Relevant experience would be a great advantage.

MRC conditions of service, U.S.S. benefits, salary in the range of £11070-£15720. Applications with curriculum vitae and names of 2 referees should reach Roger Lawrence at the above address by 30th April.  
(8946)A



The Universities of Strasbourg, Freiburg im Breisgau, Karlsruhe and Basel have created a trinational curriculum in Biotechnology. The

### Faculty of Sciences of the University of Basel

has assumed the responsibility for the teaching of *Microbiology* in this curriculum, and for this purpose a new

### Professorship in Biotechnology

has been funded for January 1, 1990.

The duties will include:

- ☆ Organisation and execution of Microbiology teaching in the new curriculum with the help of the existing infrastructure.
- ☆ Research on an aspect of Microbiology relevant to Biotechnology.
- ☆ Participation in advanced level teaching at the Biozentrum of the University of Basel.

The University will provide core support in the form of salaried positions, equipment and running expenses.

Applications including a curriculum vitae, list of publications, reprints of about five representative publications, a short overview of past and planned research activities and a description of teaching experience should be submitted before June 1, 1989, to the

**Dean of the Faculty of Sciences**

at the following address:

**Dekan der Philosophisch-Naturwissenschaftlichen Fakultät,  
Klingelbergstrasse 23, CH-4056 Basel, Schweiz.**

Further information on the curriculum in Biotechnology and on the Biozentrum is available on request. (W6044)A

## DIRECTOR NORTHWESTERN UNIVERSITY INSTITUTE FOR NEUROSCIENCE

Applications and nominations are invited for the Directorship of the newly formed Institute for Neuroscience at Northwestern University. This is a University wide institute whose members come from 10 departments spread across four schools and several teaching hospitals. Current Institute membership exceeds 50 faculty. The Director will be expected to oversee the University-wide commitment to further expand faculty and research activities in the neurosciences. The Director will become the incumbent of an endowed Chair in Neuroscience. Candidates are expected to be internationally recognized leaders in the field of Neuroscience.

Those interested should submit an up to date curriculum vitae along with the names of four references to:

**Dr. Robert D. Goldman  
Chairman**

**Institute for Neuroscience Search Committee  
Department of Cell Biology and Anatomy  
303 East Chicago Avenue  
Chicago, Illinois 60611-3008**

*Northwestern University is an Affirmative Action  
Equal Opportunity employer.*

(NW3585)A

## Postdoctoral Research/Immunology Department of Medicine and Graduate Program in Immunology Tufts University School of Medicine

Postdoctoral fellowship available to study systemic autoimmune disease in mice and humans: Organization/expression of genes encoding pathogenic autoantibodies & the structure/specificity of T cell receptors used by pathogenic autoantibody-inducing T cells (see: J. Immunol. 138, 128-137, 1987; J. Exp. Med. 165, 1252-1268, 1987; PNAS. 84, 6850-6853, 1987; J. Immunol. 140, 2215-2224, 1988).

Experience in molecular biology techniques preferred. Send résumé and names of three references to: **Dr. Syamal K. Datta, Department of Medicine, Box 52, Tufts University, New England Medical Center, 750 Washington Street, Boston, MA 02111.** (NW3595)A

## KENT



## Research Fellow in Molecular Biology

Applicants are invited for a post of Research Fellow to undertake a study of the mechanism of protein synthesis in the fungal pathogen *Candida albicans* using a range of molecular biology techniques. Applicants should have a PhD or be in the process of submitting for a PhD, in the areas of molecular biology, genetics or biochemistry. The successful applicant will join a well established group at the University of Kent currently studying various aspects of protein synthesis in yeasts, under the direction of Dr M F Tuite.

The project is funded by Glaxo Group Research. An appointment will be made on the RA1A scale (£9,865 to £15,720 per annum) for two years in the first instance, with the possibility of a further appointment for one year. The starting date is negotiable.

Enquiries about the post can be made to Dr M F Tuite on (0227) 764000, ext 3699.

Application forms and further particulars may be obtained from **Mr J E Reilly, Secretary of Faculties and Deputy Registrar, The Registry, University of Kent at Canterbury, Kent CT2 7NZ** quoting reference A89/38. Closing date: 5 May 1989.

*The University of Kent is an equal opportunities employer.* (8974)A

## THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following position:

**NORTH AUSTRALIA RESEARCH  
UNIT**

**POSTDOCTORAL FELLOW  
RESEARCH FELLOW/  
SENIOR RESEARCH  
FELLOW**

Task: to initiate work on policy problems associated with the use, development and conservation of the natural resources, especially savanna land, of Northern Australia.

Qualifications: A PhD in economics, agricultural economics, or natural resource management or experience in the analysis of resource related problems is essential. Postgraduate research experience in the relationship between social and ecological sciences would be an advantage.

Post: Full time research, available January 1990, location Darwin, Australia. Secondment from other institutions considered.

Details: A short statement of proposed research is to be submitted with the application. Further details from the Executive Director, NARU, PO Box 41321, Casuarina, NT 0811, Phone (089) 275688, FAX (089) 450752.

Closing date: 30 June 1989. Ref PA 30.3.3.

**SALARY** Senior Research Fellow; A\$42,582-A\$50,619 p.a. Research Fellow A\$30,737-A\$40,100 p.a.; Postdoctoral Fellow Grade 1 (fixed point); A\$26,617-A\$30,360 p.a. **APPOINTMENT:** Senior Research Fellow/Research Fellow up to three years, possibility of extension to five years; Postdoctoral Fellow normally two years, possibility of extension to three years. **APPLICATIONS** should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from Appointments (36235), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

**THE UNIVERSITY IS AN  
EQUAL OPPORTUNITY EMPLOYER** (W6047)A



The University  
of Sydney, Australia

## POSTDOCTORAL FELLOW/ RESEARCH FELLOW NEUROCHEMISTRY/ NEUROPHARMACOLOGY

Reference No. 12/04

DEPARTMENT OF PHARMACOLOGY

Higher degree graduate in pharmacology, physiology, biochemistry or a related discipline to work on a NH&MRC Program Grant "Neurochemical pharmacology of amino acid transmitters in the nervous system". We are particularly interested in a person able to lead investigations on the interactions of steroids with GABA neurotransmitter systems and/or the roles of amino acid neurotransmitters in various neurological disorders. The position is available for 1 year in the first instance with possible extension until the end of 1991. The level of appointment and responsibility will depend on the appropriate qualifications and experience of the appointee. Further information from Professor Graham Johnston or Dr Robin Allan, (02) 692 2408, FAX (02) 692 3868.

**SALARY:** Postdoctoral Fellow \$A26,617-\$A29,611 per annum.  
Research Fellow \$A30,737-\$A40,100 per annum.

**METHOD OF APPLICATION:** Applications, quoting reference no., including curriculum vitae, list of publications and the names, addresses and FAX numbers of three referees, to the Registrar, Staff Office, University of Sydney, NSW 2006, Australia, by 24 April, 1989. (W6048)A

*Equal employment opportunity is University policy*

## UNIVERSITY OF SURREY HIGH PRESSURES AND LOW DIMENSIONAL SYSTEMS Research Fellow

A co-operative SERC project between physics groups in six universities (Cambridge, Edinburgh, Imperial College (London), Nottingham, Oxford and Surrey) and the SERC High Pressure Facility at STC Technology Ltd Harlow, and supported in part by STC funding, requires the recruitment of a post-doctoral research fellow: a physicist or physical chemist with some understanding of compound semiconductors, their band structure and optical and photoluminescent properties. The successful candidate would develop measurement techniques at very high pressures based mainly on diamond anvil cells, and transfer these to laboratories collaborating in the SERC Low Dimensional Systems Initiative. This is challenging work requiring creative insight combined with considerable practical skill.

The research fellow will be attached to the University of Surrey but would work at the SERC High Pressure Facility at Harlow (the only SERC Facility in an industrial research centre, and a small unit used by researchers from all over the country). The appointment will be for a period of up to three years. Salary will be on the Research and Analogous Range 1A scale according to age, experience and qualifications. Further information can be obtained from Professor C H L Goodman, Director SERC High Pressure Facility, STC Technology Ltd., London Road, Harlow, Essex CM17 9NA, tel 0279 29531 ext 3308.

Applications in the form of a curriculum vitae (3 copies) including the names and addresses of two referees, should be sent to the **Personnel Office (JLG), University of Surrey, Guildford GU2 5XH**, quoting ref 833N by 5 May 1989. (8986)A

## University of Bristol SCHOOL OF VETERINARY SCIENCE CHAIR IN VETERINARY MEDICINE

Applications are invited for the Chair in Veterinary Medicine which has fallen vacant following the resignation of Professor FJ Bourne. Candidates, who must have a veterinary qualification, should submit applications, including the names of three referees, not later than 31 May 1989. Full particulars of the appointment may be obtained from the **Registrar, University of Bristol, Senate House, Bristol BS8 1TH**. (8980)A

## POSTDOCTORAL RESEARCHERS

The "Biomolecular Mechanisms" section of the Los Alamos National Laboratory has openings for postdoctoral fellows. Research conducted in this program includes:

- 1) Studies on the vertebrate rod, a single photon detector that can regulate its sensitivity over a wide dynamic range. We are examining the fine control of those gene products which regulate the photon-sensitive membrane current in this photoreceptor cell.
- 2) Studies on the microangiopathy of diabetes, a primary cause of human blindness and renal failure. We are examining the regulation of sodium transport and cell volume in mammalian erythrocytes and the reversible impairment of Na<sup>+</sup> transport and red cell swelling found in hyperglycemic states.
- 3) The differential excretion of glycated and unmodified albumins by the mammalian nephron is correlated with age. We are examining age related changes in the biochemical composition and physical properties of selected basement membrane structures.

Applicants should have a strong background in protein chemistry and enzyme regulation, a doctoral degree obtained in the past three years, and must qualify for a Department of Energy "Q" security clearance. Postdoctoral appointments are for one year, renewable for a second year. The salary range is \$33,640-\$35,445 per year. Please send a curriculum vitae to Dr. Mark W. Bitensky, Senior Fellow, ADR, MS D434, Los Alamos National Laboratory, Los Alamos, NM 87545. (505) 667-9420.

To apply formally, send resume, employment application, graduate and undergraduate transcripts and three letters of reference to Carol M. Rich, Human Resources Development Division, PD-89-060, Los Alamos National Laboratory, Los Alamos, NM 87545.

Affirmative Action/Equal Opportunity Employer

University of California

(NW3601)A

# Los Alamos

## DEPARTMENT OF PHARMACEUTICAL SCIENCES CONTRACT RESEARCH ASSISTANT/ RESEARCH FELLOW

Applications are invited for the above appointment to work with Dr. P. J. Hanson on a project funded by the Medical Research Council to investigate the signal transduction mechanisms involved in the inhibition of gastric acid secretion by epidermal growth factor.

Applicants should possess, or expect to attain a 1st or 2(i) honours degree in biochemistry, cellular physiology or a related discipline. Candidates for appointment at Research Fellow level will be expected to have undertaken a PhD degree. The position is available for three years from 1 October, 1989.

**Starting salaries** (according to age and experience):

Research Fellow (Research Grade 1A): £9,865 to £15,720 p.a.

Research Assistant (Research Grade 1B): £8,675 to £11,680 p.a.

Application forms and further particulars may be obtained from the Personnel Officer (Academic Staff), quoting Ref:897/6, Aston University, Aston



Triangle, Birmingham B4 7ET. Tel: 021-359 0870 (24 hour answer-phone). Closing date for the receipt of applications is 4th May, 1989.

ASTON UNIVERSITY

(8944)A

## POSTDOCTORAL AND RESEARCH ASSOCIATE POSITIONS MOLECULAR VIROLOGY/BIOLOGY

Postdoctoral and research associate positions are available (beginning Summer, 1989) to participate in ongoing research on the molecular biology, virology, and pathogenesis of the simian and feline immunodeficiency viruses (SIV and FIV). The applicant would be a member of an NIH-funded multidisciplinary group developing animal models for AIDS. Newly constructed laboratory space (including BL-3 facilities) is available and is equipped with state-of-the-art instrumentation (including DNA synthesizer, automated DNA sequencer, PCR cyclers, etc).

Applicants should possess a Ph.D., M.D. or D.V.M. degree and have demonstrated skills in basic molecular biology techniques. Salary will be commensurate with experience and expertise. Interested candidates should send a curriculum vitae and the names of three references to:

**Dr. Philip R. Johnson**

**Division of Molecular Virology and Immunology,  
Department of Microbiology**

**Georgetown University School of Medicine  
NIH/Twinbrook II**

**12441 Parklawn Drive, Rockville, MD 20852**

*Georgetown University is an Equal Opportunity/  
Affirmative Action Employer (NW3587)A*

## UNIVERSITY OF MAINE POTATO BIOTECHNOLOGY PROGRAM

### TWO POSTDOCTORAL RESEARCH ASSOCIATES

Applications are invited for two postdoctoral scientists to join a group working on the molecular biology of disease resistance genes in potato. The group works in well equipped laboratories and specialist training is available in a wide range of techniques.

The appointments are for two years with extension contingent upon continued funding. Experience with recombinant DNA techniques or plant transformation and tissue culture is desirable. Salary commensurate with experience.

Send curriculum vitae and three letters of reference by 5/15/89 to **Dr. Stellos M. Tavantzis** or **Dr. Michael E. Vayda**, 178 Hitchner Hall, University of Maine, Orono, ME 04469, U.S.A.

An EEO/AA institution

(NW3588)A

## UNIVERSITY COLLEGE OF SWANSEA

### RESEARCH ASSISTANT

Applications are invited for the vacancy of Research Assistant, in the School of Biological Sciences, to work on the mechanism of immunorecognition in insects with Professor N A Ratcliffe and Dr A F Rowley. The project is funded by S.E.R.C. and will include raising monoclonal antibodies to insect cells and the subsequent testing of these molecules. Applicants should have a degree in biology, biochemistry or immunology and preferably some further research experience.

The post is tenable for up to 3 years, at a commencing salary of up to £10,460 per annum, plus USS/USDPS benefits.

Informal enquiries may be made directly to Dr A F Rowley (0792) 295455, but further particulars and application forms (2 copies) must be obtained from the **Personnel Office, University College of Swansea, Singleton Park, Swansea SA2 8PP**, to which office they should be returned by **Friday 5 May 1989**. (8988)A

## POSTDOCTORAL POSITION

### Membrane Biophysics

Areas of interest include lipid polymorphism and its relationship to membrane fusion, the regulation of membrane-bound enzymes and multi-drug resistance.

Highly qualified individuals please send full curriculum vitae and names of two references to: **Dr. R.M. Epand, Dept. of Biochemistry, McMaster University Health Sciences Centre, 1200 Main Street West, Hamilton, Ontario, Canada, L8N 3Z5**. (NW3583)A

## ASSISTANT/ASSOCIATE PROFESSOR IN SEDIMENT GEOCHEMISTRY

The College of Oceanography at Oregon State University is seeking applicants for a tenure track position in sediment geochemistry at the Assistant or Associate Professor level. Applicants who have ongoing research programs which complement and augment one or more of the existing OSU programs in Pleistocene paleoclimatology, sedimentation and fluid circulation at convergent margins, contemporary sedimentary cycling, benthic fluxes, geochemical tracers of ocean circulation and productivity, or seawater hydrothermal systems are urged to apply. The College of Oceanography is well equipped for research in these areas with facilities for: stable isotope and noble gas mass spectrometry, XRF, XRD, AAs, electron microprobe, neutron activation, particle flux measurement, radiochemistry, and a UNIX computer network. Although the successful applicant is expected to teach, the usual teaching load is limited to one or two courses per year. Salary, a portion of which is state-supported, is commensurate with experience and academic rank. Applications including vitae, grant list, and names, addresses, and phone numbers of three references must be received by 1 May 1989. Send to: **Douglas R. Caldwell, College of Oceanography, Oregon State University, Oceanography Admin Bldg 104, Corvallis, OR 97331-5503**. Oregon State University is an AA/EEO employer, complies with Section 504 of the 1973 Rehabilitation Act, and has a policy of being responsive to the needs of dual-career couples. (NW3599)A

## UNIVERSITY COLLEGE DUBLIN

### DEPARTMENT OF GEOLOGY

Applications are invited for a temporary, academic appointment in the Department of Geology. This appointment will be for nine months from 1st October, 1989 to replace a staff member on leave of absence and will be made at the level of Assistant Lecturer. Duties will include the teaching of metamorphic petrology, geotectonics and Precambrian Geology to undergraduates in all years and some service teaching to students from other faculties.

The current salary scale for an Assistant Lecturer is Ir£10,566-Ir£16,958. Entry point on this scale will be in accordance with qualifications and experience.

Prior to application, details of application procedure should be obtained from the Secretary, University College Dublin, Belfield, Dublin 4. Telephone enquiries: 693244, ext. 1431.

The closing date for receipt of completed applications is Thursday, 11th May, 1989. (8970)A





University of Zürich  
Faculty of Medicine and  
Faculty of Natural Sciences

## FACULTY POSITION IN BIOCHEMISTRY

Applications are invited for a tenured faculty position (associate professorship, Extraordinariat) at the Department of Biochemistry. Candidates are expected to have a creative research programme in protein biochemistry and a genuine interest in the teaching of biochemistry to medical and science students.

Applications including curriculum vitae, bibliography synopsis of past and planned future research, and a record of teaching experience should be sent to the Chairman of the Search Committee, Professor Christian Bauer Physiologisches Institut der Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland.

Deadline June 15, 1989.

(W6022)A

## Postdoctoral Positions in Molecular Genetics

Applications are invited for 2 postdoctoral positions in the Molecular Genetics group of Ciba-Geigy Ltd, Basle, for a period up to three years. Candidates are expected to participate in; (i) a mouse molecular genetics program to generate mouse models for human diseases using gene transfer and homologous recombination strategies in mouse embryonic stem cells, (ii) a polypeptide growth factor program to generate gene-specific hybridisation procedures for studying the *in situ* localisation and expression of different growth factors in mammalian tissues. Candidates should have a PhD with 0 – 3 years postdoctoral experience preferably with training/expertise in mammalian tissue culture techniques (i) or histopathology (ii). Experience with recombinant DNA technology is desirable for both positions.

Ciba-Geigy is a major pharmaceutical company with an active interest and engagement in molecular biology. The Molecular Genetics section is located in the company's main research facility situated in Basle, Switzerland. Basle is a major centre for both pure and applied research in molecular biology, and provides a stimulating environment in every respect.

Applications including a curriculum vitae, a summary of relevant research background and the names of two referees should be sent under ref. "Nature 348" to Mr. H. Schmid, Ciba-Geigy Ltd, Personnel Department, P.O. Box, CH-4002 Basle. W(6043)A

## CIBA-GEIGY

**nature**  
INTERNATIONAL JOURNAL OF SCIENCE

**SCIENCE IN EUROPE**  
27th April 1989

This special feature issue of *Nature* will focus on the present pattern of science in Europe.

Vacancies, fellowships, symposia, conferences, lectures, workshops, announcements will all benefit from the extra interest generated by this truly "European" issue.

Copy Date: Friday noon: 21st April  
Call us today to place your advertisement.

London—Julie Skeet Tel: 01-872 0102 Fax: 01-240 2408	Munich—Sabine Fürst: Tel: (089) 26 50 32 Fax: (089) 26 93 24	Paris—Clare Newell: Tel: (1) 40 53 03 39
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## UNIVERSITY OF SUSSEX SCHOOL OF BIOLOGICAL SCIENCES RESEARCH OFFICER/RESEARCH FELLOW

A research fellow (preferably post-doctoral) is required to join a project on the mechanisms involved in the repair of DNA replication errors in human cells. The work involves the construction and transfection of viral vectors as well as DNA sequencing and possibly some *in vitro* work.

The person should preferably have experience of, or a strong desire to learn either molecular biology or tissue culture. The post is funded by the CRC for 2½ years and is available after 1st May, 1989. Salary will be on the scale £8,675-£11,680 p.a. or £9,865-£15,720 p.a., according to qualifications and experience.

Applications should be made to Dr. J.F. Burke, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. Tel (0273) 678308.

AN EQUAL OPPORTUNITY EMPLOYER

(8967)A

## Texas A&M University Department of Entomology

**Postdoctoral Research Associate** position available immediately for 2 years at Texas A&M University, Department of Entomology, College Station, Texas to study molecular biology of gene expression using recombinant forms of a genetically engineered baculovirus. Successful applicant should be training in recombinant DNA techniques; experience in cell and tissue culture also required for the conduct of the research. Salary \$22-25,000. Send curriculum vitae and names of three references preferably by May 15, 1989 to Dr. Max D. Summers, Distinguished Professor, Department of Entomology, Texas A&M University, College Station, Texas 77843. An equal opportunity employer. (NW3600)A



## Northern Ireland Civil Service

### HEAD OF AQUATIC SCIENCES RESEARCH DIVISION (SENIOR PRINCIPAL SCIENTIFIC OFFICER)

**Salary: £21,633-£28,170**

**Closing Date: 5 May 1989**

**Reference: SB 38/89**

**Tel: Ext. 2304**

#### DEPARTMENT OF AGRICULTURE (NORTHERN IRELAND)

Applications are invited for the Head of Aquatic Sciences Research Division, a newly organised DANI Research Division. The successful candidate will be responsible for the management of some 40 scientific staff and all aspects of their research and technical work in the Aquatic Sciences field. He/she will also be responsible for the line management of all aspects of the research division. The principal task will be to initiate and maintain an innovative basic and strategic research programme of direct relevance to DANI's responsibilities for agriculture and fisheries. He/she will also be responsible for the conduct of other scientific and technical programmes in this area.

The postholder will also manage, via a non-government agency, DANI's marine research vessel and its scientific programme around the coasts of Britain and Ireland. He/she may be required to lecture to under-graduates in the Faculty of Agriculture—Queen's University, Belfast.

The successful candidate must possess a PhD in a relevant scientific discipline and have a proven track record of research in marine/fresh water aquatic science.

In addition he/she must have demonstrable ability and experience in managing staff, finance and other resources, managing multi-disciplinary scientific teams and attracting outside funding. Substantial sea-going experience on medium to large marine research vessels and a sound knowledge of on-board scientific techniques, working practices and associated management procedures would be highly desirable. Experience in an international context at research and/or other levels would be an advantage.

Starting salary will be in the range £21,633 to £28,170 with further increments payable, depending on performance, up to a maximum of £32,826.

The Civil Service Commissioners may decide to interview only those applicants who appear, from the information available, to be most suitable in terms of experience and academic qualifications.

Please write to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR or telephone Dundonald 4567 for an application form (using the extension number indicated and quoting the Job Reference). Completed forms must be returned to arrive not later than the closing date stated. (8966)A

The Northern Ireland Civil Service is an Equal Opportunity Employer. Its posts are open to both men and women and applications are welcome from disabled persons.



# nature

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of science

### UNIVERSITY OF BIRMINGHAM DEPARTMENT OF SURGERY Queen Elizabeth Hospital

#### CANCER RESEARCH CAMPAIGN FUNDED RESEARCH ASSOCIATE/RESEARCH FELLOW

Applications are invited from scientists at post-graduate or post-doctoral level interested in an investigation into the novel effects of bile acids and their metabolites on the growth and steroid receptor activity of human breast cancer cells and tissues.

The project is part of a larger programme of research currently being established in the Department of Surgery into the role of bile acids, steroids and lipids in the biology of human cancers. The successful candidate will have the opportunity to make a major contribution of the bile acid/breast cancer study and to play a key role in the future direction of the groups research programme. Experience in cell and tissue culture techniques would be an advantage but is not essential.

Appointment is for up to two years on the Research Associate 1B (£8675 – £11680) or Research Fellow 1A (£9865 – £15720) Scale with superannuation.

Informal enquiries to Dr. P. R. Baker, Tel 021 414 4103.

Application forms with further particulars from **Senior Assistant Registrar, The Medical School, B15 2TJ by 5 May 1989.**

**Quote Ref. RF/Surg/PB4**

**AN EQUAL OPPORTUNITIES EMPLOYER**

(8947)A

### UNIVERSITY OF ST ANDREWS Department of Biology and Preclinical Medicine LECTURESHIP IN BIOLOGY

Applications are invited for the above post from candidates with a strong research record in any area of Plant Biology. Candidates interested in Plant Molecular Biology and/or Pathology will be welcome provided that they have a broad background in Plant Biology.

The appointment is tenable from 1st October 1989 and the salary will be on either the Lecturer Grade A scale £9,260 to £14,500, or Grade B scale £15,105 to £19,310 per annum, initial appointment probably on the Grade A scale.

Further particulars and application forms are available from the **Director of Personnel Services, The University, College Gate, St Andrews, Fife KY16 9ANJ, to whom applications should be submitted not later than 25th April 1989.** (8906)A

### UNIVERSITY OF LIVERPOOL Chair of Veterinary Anatomy

Applications are invited for the Chair of Veterinary Anatomy. The Chair will be held in the Department of Veterinary Preclinical Sciences, in the Faculty of Veterinary Science. Preferred applicants will have leadership experience in research and teaching within morphological sciences appropriate to veterinary medicine.

The salary will be within the range approved for non-clinical professorial salaries, currently not less than £24,499 per annum.

Applications, together with the names of three referees, should be received not later than 31 May 1989, by **The Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool, L69 3BX,** from whom further particulars may be obtained.

**Quote Ref. RV/322.**

**An Equal Opportunity Employer**

(8998)A

### UNIVERSITY OF STIRLING LECTURER IN CHEMISTRY

Applications are invited for the post of Lecturer in Chemistry within the newly formed School of Molecular and Biological Sciences. Applicants should have a good background in Physical Chemistry and preference will be given to those who can show that their area of research would allow an interface with the Biological Sciences. The appointment will be on the Grade A scale, currently £9260-£14500 (under review).

Applications including a c.v. and the name of three referees should be sent by 5 May 1989 to **The Staff Office, University of Stirling, Stirling FK9 4LA; Tel: 0786, 73171, Ext 2314,** from whom further particulars may be obtained. (8992)A

THE AFRC INSTITUTE OF  
HORTICULTURAL  
RESEARCH

East Malling

Higher Scientific Officer

A plant Physiologist/Biochemist (HSO Band I) is required to develop a programme on stress physiology under the guidance of the Head of Division (Dr H.G. Jones). The objectives of the work are to identify the biophysical and biochemical mechanisms involved in plant response to and adaptation to environmental stress. The appointee would act as local co-ordinator of the stress physiology group at East Malling.

Qualifications: First or Upper Second class honours degree in an appropriate subject plus at least 2 years' relevant postgraduate experience, or equivalent. Experience in biophysical or biochemical research on aspects of plant stress physiology. Experience in water relations and photosynthesis (including fluorescence) research or plant developmental physiology an advantage.

Salary: In range £10,026-£13,460.

Further details and application form from the Personnel Officer, Institute of Horticultural Research, Bradbourne House, East Malling, Maidstone, Kent ME19 6BJ. Closing date 4th May 1989.

The AFRC is an Equal Opportunities Employer (9003)A

# RHONE-POULENC SANTE

The health division of France's principal chemical group

Invites applications from experienced molecular biologists for three RESEARCH SCIENTIST posts in its newly built Biotechnology Institute near Paris.

## Streptomyces molecular genetics

This position requires a Ph.D. in bacterial molecular biology as well as a minimum of 3-4 years' post-doctoral experience in the Streptomyces field. Familiarity with recombinant DNA technology in Streptomyces microorganisms, as well as experience in cloning genes involved in secondary metabolism, is essential. Réf. 50425/A.

## Mammalian cloning technology

Qualified candidates for this position will have a Ph.D. and good post-doctoral experience in the cloning and analysis of mammalian cDNAs or genes. A proven record of achievement using up-to-date DNA cloning strategies (construction of cDNA libraries in various vectors: screening by oligonucleotide hybridization, immunodetection or expression, subtractive hybridization...) is essential. Réf. 50425/B.

## Transgenic animals

This position requires a Ph.D. level in cellular and molecular biology and a good post-doctoral experience in the production and use of transgenic animals. The candidate should be able to develop a small unit for the production and genetic analysis of transgenic animals. Réf. 50425/C.

Please send your full resume quoting the reference to Media-System, 6/8 impasse des Deux Cousins, 75849 Paris Cedex 17, France.

(W6053)A



## KING'S COLLEGE LONDON Research Associate

Applications are invited from scientists with experience in the application of physical biochemistry and molecular genetics to the study of DNA binding proteins and mechanisms of gene regulation. This position in Professor Gould's group, available for two years from July 1989, will involve studies on the interactions between nucleosomes on specific gene sequences and known regulatory proteins. Annual inclusive salary up to £17,370 according to age and experience.

Applications (*curriculum vitae* and the names and addresses of two referees) should reach **Mr D M Drummie, Biophysics Laboratories, King's College London, 26-29 Drury Lane, London WC2B 5RL**, by 27 April 1989.

(9000)A

## UNIVERSITY OF NEWCASTLE UPON TYNE CHAIR OF BIOCHEMISTRY

Applications are invited for the Chair of Biochemistry which has recently become vacant on the retirement of Professor K. Burton, F.R.S.

The person appointed will be expected to provide academic leadership in research and teaching within the Department of Biochemistry and Genetics. The Department is in the Faculty of Science, is a member of a Cross-Faculty School of Biomedical and Biomolecular Sciences and is located in the new Medical School. This offers opportunities for collaboration within a broad area of research.

Salary will be at an appropriate point on the Professional salary scale. Further particulars may be obtained from the **Deputy Registrar, the University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU** with whom applications (15 copies), giving the names of three referees, should be lodged not later than 26th May 1989. (*Applicants from outside the U.K. may submit one copy only*). (8987)A

## ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

### Department of Paediatrics and Neonatal Medicine Jerry Lewis Muscle Research Centre POSTGRADUATE RESEARCH ASSISTANT

To work on the structure and regulation of potassium channels in skeletal muscle, using specific toxins, monoclonal antibodies and cDNA probes. Experience in these areas and/or with membrane biochemistry, FPLC and tissue culture and advantage, but not essential. The post is available from October for three years and is funded by the Muscular Dystrophy Group of Great Britain. Applicants should either hold or expect to gain a good honours degree. The successful candidate will, in appropriate circumstances, be eligible to register for a higher degree. Salary: up to £9260 plus London Allowance of £1650.

For informal enquiries, please contact **Dr Peter Strong (01-743 2030 ext 2124)**. Application forms and further information from the **Personnel Office, Royal Postgraduate Medical School, London W12 0NN (tel: 01-740 3204) quoting reference ANW8**. Closing date 8 May 1989. (9001)A

## University of Bristol TEMPORARY LECTURER IN ANATOMY

Applications are invited for a Temporary Lectureship in Anatomy for the session 1989/90. Applicants should have special interests in embryology and reproductive biology. The post is funded by the Wellcome Trust to provide research leave for Dr. Claire Wathes and the appointee will be required to teach medical and veterinary students. Salary in the range £12,150-£13,365 per annum (under review).

Applications (in duplicate) stating age, qualification, and including the names and addresses of three referees, should be sent by 5 May 1989 to **The Registrar, University of Bristol, Senate House, Bristol BS8 1TH** from whom further particulars may be obtained. Please quote reference TLJ. (8994)A



## Overseas Development Natural Resources Institute

# Ruminant Nutritionalists

## Chatham Based

The Animal Products and Feed Department of the ODNRI is concerned with post-harvest aspects of fish, meat, hides and skins, dairy products and animal feeds.

Two posts are available to work on developing programmes aimed at improving livestock production through improvements in nutrition. This will involve close collaboration with a wide range of professional staff and will entail co-operation with centres overseas. The work involves long and short assignments in developing countries.

For both posts you should have a good honours degree and a higher degree is desirable.

For the higher post (Ref: S/F/677) you will probably have 10 years' or more relevant experience including extensive cattle production in developing countries.

For the second post (Ref: SB/66/JD) you must have at least 2 years' relevant experience. Experience of applied nutrition of sheep, goats or cattle in developing countries would be an advantage.

Starting salary is in the range £10,505-£22,605 (depending on qualifications and experience) with further increments, depending on performance, up to £26,955 (for the higher post). In addition posts in London attract Inner London Weighting of £1750 per annum.

The Overseas Development Natural Resources Institute will be relocating to Chatham, Kent in late 1989. Prior to that date employment will be in London or at Culham (Oxfordshire).

For further details and an application form (to be returned by 28 April 1989) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours). Please quote appropriate reference number.

The Civil Service is an equal opportunity employer

*Scientific*  
CIVIL SERVICE

(9007)A

nature

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## INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH EDINBURGH

Two IMMUNOLOGISTS are required, one as project leader and the other as research assistant, to study cellular immune responses in cattle to the protozoan parasite *Theileria annulata*. The research is directed towards production of a molecular vaccine and will concentrate particularly on the identification of T cell epitopes on relevant parasite antigens. An active programme of research on the MHC and its role in T cell immunity is ongoing in the department and the Institute is very well equipped for both cellular immunological and molecular biological research.

The appointment will be at Senior or Higher Scientific Officer levels for the project leader and Scientific Officer for the research assistant.

Qualifications for the SSO/HSO are a Ph.D with at least three years relevant post graduate experience in Immunology; for the SO post a 1st or 2.1 honours degree in a relevant subject. The SO may be able to study for a Ph.D.

The project is financed under the EEC programme of Science and Technology for Development will be for 3 years and will involve collaboration with workers in Morocco, the Centre for Tropical Veterinary Medicine, University of Edinburgh and the Wellcome Unit of Molecular Parasitology in Glasgow.

Salary: In scale SSO £12445-£17032. HSO £10026-£13460.  
SO £8574-£10994.

Further information can be obtained from Dr. R.L. Spooner or Dr. E.J. Glass at the Department of Immunogenetics. Telephone 031-667-6901. Application forms from the **Personnel Officer, Mrs. L. Hunter at the Institute of Animal Physiology and Genetics Research, Roslin, Midlothian, EH25 9PS**. Completed application forms must be returned by 5th May 1989. (8963)A

## Senior Technician Grade 8C (Molecular Biology/Genetics and Biochemistry)

Salary Scale £13,575 - £14,736 inclusive of London Allowance

This is a newly created post in the School of Biological and Health Sciences responsible for providing a state-of-the-art service and contributing to the development of improved methods in experimental biochemistry, gene manipulation and molecular biology. Applicants should have a degree or equivalent qualification and relevant experience.

Please telephone 01-580 2020 ext 2136 (answerphone) for an application form and further details or write to Personnel Department, PCL, 309 Regent Street, London W1R 8AL quoting Ref 10402. Closing date for the receipt of completed application forms is 24 April 1989.

PCL is an Equal  
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**PCL**  
THE POLYTECHNIC  
OF CENTRAL LONDON

(8982)A

## INSTITUTE OF PSYCHIATRY DEPARTMENT OF NEUROLOGY POST DOCTORAL RESEARCH ASSISTANT

Applications are invited for a Research Assistant to study the role of monoamines in ischaemic and excitotoxic brain damage. The post is funded by the MRC for 3 years. The project involves microdialysis in vivo with HPLC analysis of amino acids and monoamines, and histological analysis of outcome. Candidates with relevant experience should contact Prof B Meldrum on 01 703 5411 (ext 3398, 3399).

Starting salary on the 1A scale up to £11680 pa plus £1650 London Allowance.

Applications in the form of a curriculum vitae and the names of two referees should be sent to the **Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF** quoting reference BM.

Closing date: 28th April 1989.

(8971)A

**UNIVERSITY OF NOTTINGHAM**  
**School of Agriculture**  
**Department of Physiology and Environmental Science**

**RESEARCH TECHNICIAN GRADE 3**

Applications are invited from suitably qualified candidates to work on a project financed by Agricultural Genetics Company of Cambridge on biological control of plant diseases using bacterial inoculations. A qualification in microbiology is necessary and experience in fermentation technology would be an advantage.

Commencing as soon as possible this post will be offered initially for a period of 12 months. Salary on a scale £6,636 to £6,871 according to age, qualifications and previous experience. Minimum qualification ONC, TEC or equivalent.

Application forms obtainable from the Administrative Assistant, University of Nottingham, School of Agriculture, Sutton Bonington, Nr Loughborough, Leics LE12 5RD. Telephone Nottingham 484848 Ext 8302. Please quote Post Ref. 89/08. (8953)A

**POSTDOCTORAL POSITION: Neurobiology of Disease**

The Department of Pathology at U.W.O. invites applications from individuals interested in neurobiology of disease with a background in structure and function of biological peptides, including molecular biology techniques and monoclonal antibody production. The project involves the study of a neuronal protein related to resistance to excitotoxic (epileptic) injury. This protein is abundant in neurites of plaques in Alzheimer disease.

Send curriculum vitae and names of three references to: **Dr. David Munoz, Department of Pathology, University of Western Ontario, Health Sciences Center, London, Ontario, Canada N6A 5C1** (NW3598)A

**AQUATIC TOXICOLOGIST**

World-renowned environmental testing laboratory seeks experienced aquatic toxicologist to manage a large, newly constructed commercial aquatic toxicology laboratory. Successful candidate must have doctorate or equivalent and be familiar with the design, conduct and reporting of freshwater and marine tests. Preference will be given to those with demonstrated management skills and a working familiarity with good laboratory practices. Excellent writing skills a must. Position open immediately. Excellent benefits. Salary dependent upon experience. Send résumé to: **Aquatic Programs, Wildlife International Ltd., 305 Commerce Drive, Easton, MD 21601.** (NW3584)A

**DEPARTMENT OF VETERANS AFFAIRS**

**VETERANS HEALTH SERVICES AND RESEARCH ADMINISTRATION**

**DIRECTOR MEDICAL RESEARCH SERVICE**

The Department of Veterans Affairs invites applications for the position of Director, Medical Research Service. The Medical Research Service currently has a budget of about \$200 million and supports biomedical and behavioural sciences research of over 2500 VA scientists, 70 percent of whom are physicians. It is strongly committed to support of patient-oriented research across the entire spectrum from basic science to clinical applications of new knowledge. The Director is responsible for policy development, peer review management of all investigator-initiated research studies and budget formulation and distribution. Candidates are expected to be nationally recognized scientists and they must have an established record of scholarly achievement. The position is only open to U.S. citizens who hold an M.D. degree. Salary is \$75,500 with additional special pay up to \$24,000.

Qualified applicants are invited to send a curriculum vitae, bibliography and the names, addresses and telephone numbers of three respondents by 15 May 1989 to: **Richard J. Greene, M.D., Ph.D., Assistant Chief Medical Director for Research and Development (15), Department of Veterans Affairs, 810 Vermont Avenue, N.W., Washington, D.C. 20420.**



**Veterans Administration**

(NW3541)A

**THE UNIVERSITY OF LEEDS DEPARTMENT OF PATHOLOGY PREDOCTORAL RESEARCH ASSISTANT/POSTDOCTORAL RESEARCH FELLOW**

Applications are invited for the above post available as soon as possible for a fixed period of three years for work on identification of oncogene abnormalities in cervical cancer which may be used as a screening test, involving the use of PCR, RNaseA digestion and other molecular biological techniques.

A good honours degree in biochemistry is required. Candidates with a PhD in molecular biology may be appointed as a Research Fellow on R/A IA.

Salary for Research Assistant on R/A Grade IB (£8675-£11680) or Research Fellow on Grade IA (£9865-£15720), according to qualifications and relevant experience.

Informal enquiries may be to Dr Philip Quirke or Mrs J Fearnley (tel (0532) 333399/87).

Application forms and further particulars may be obtained from and completed applications forwarded to the Registrar, the University, Leeds LS2 9JT (tel (0532) 333963 — direct line), quoting reference no. 102/40. Closing date for applications 4 May 1989. (8958)A

**RESEARCH ASSOCIATE (BIOCHEMIST)**

with 5 years of postdoctoral training to study flavonoids in cell activation and chemoprevention. Ph.D. biochemistry/enzymology training in polyphenol metabolism. Experience in cell and organelle isolation, membrane enzymes and purification/characterization, lipid peroxidation, HPLC, enzyme induction/xenobiotic metabolism, carcinogen-DNA binding, carcinogen metabolite characterization, radioisotope handling and writing grant proposals. Candidate expected to develop independent research program on flavonoids. Salary: to \$27,500. Send résumé and three reference letters to: **Director, Allergy Division, SUNY/Bufalo, Buffalo General Hospital, 100 High Street, Buffalo, NY 14203. Affirmative Action/Equal Opportunity Employer.** (3608)A

**ASSISTANT PROFESSOR**

Full-time (9-month), tenure-track position, beginning fall 1989. General and applied microbiology, and general biology for department majors. Competence in immunology is desirable. Ph.D. required. Send curriculum vitae and names and addresses of references to: **I. Gepner, Chair, Department of Biology, Monmouth College, West Long Branch, NJ 07764. Equal Opportunity Employer; women and minorities are encouraged to apply.** (NW3592)A

**CSIRO**

Division of Soils

Adelaide - Australia

**PLANT PATHOLOGIST**

The Job: Development of biological methods to control plant root diseases.

Duties include:

- Evaluating the ability of soil microorganisms to control seedling damping-off diseases in bedding plants;
- Conducting tests to assess microorganisms for control of seedling root diseases of legumes;
- Liaison with industry;
- Supervision of part-time Technical Assistant.

The Person: A doctoral degree specialising in plant pathology or microbiology essential.

Tenure: 15 months from June 1989.

Salary: A\$31,006 p.a. to A\$34,279 p.a.

Information: Mrs J. Clark or Dr M. Ryder. Phone (08) 274.9311. Fax: (08) 338.1636.

Application: Full C.V., including 2 professional referees, to: The Chief, CSIRO Division of Soils, PMB No. 2, Glen Osmond, S.A. 5064 by 15 May, 1989.

CSIRO is an equal opportunity employer (W6051)A

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## HUGH KNOWLES CHAIRS AUDIOLOGY AND HEARING SCIENCE NORTHWESTERN UNIVERSITY

Northwestern University announces the establishment of two endowed chairs, the **Hugh Knowles Chair in Audiology** and the **Hugh Knowles Chair in Hearing Science**. Each Chair, funded by a generous gift from Knowles Electronics, Inc., will be filled by faculty of distinguished scholarly achievement or unusual promise and developing international reputation. Applicants for the Audiology Chair should be Ph.D. scholars with an interest in auditory system function, having quantitative background (e.g., bioengineering, computer science or psychoacoustics). Applicants for the Hearing Science Chair should be Ph.D. or M.D. scholars with accomplishments in basic or clinical neuroscience and an interest in either higher nervous system function or neurobiology of the peripheral auditory system. These positions will be filled at either the full or associate professor level. Generous start-up funds, laboratory space and a competitive salary are provided.

Applications and nominations are invited. Review of applications will begin immediately, but these positions will remain available until filled. Please send curriculum vitae along with a letter of interest to:

**Chair, Search Committee  
Audiology and Hearing Impairment Program  
Northwestern University  
2299 Sheridan Road  
Evanston, IL 60208**

Northwestern University is an EO/AA Employer. Hiring is contingent upon eligibility to work in the United States.

(NW3605)A

## ASSISTANT PROFESSOR POSITIONS CELLULAR NEUROBIOLOGY AND SYSTEMS NEUROBIOLOGY DEPARTMENT OF PSYCHOBIOLOGY UNIVERSITY OF CALIFORNIA, IRVINE

Applications are invited for two tenure-track positions at the level of assistant professor in cellular neurobiology and systems neurobiology. Preference will be given to applicants who have demonstrated expertise in research areas of interest to the Department. The Department of Psychobiology emphasizes interdisciplinary approaches to the study of neural plasticity and behavior. The current major research themes of the Department are development, reorganisation following injury and disease, and learning and memory. Please submit by 15 August 1989, curriculum vitae, description of research interests, and three confidential letters of recommendation to: **Chair, Department of Psychobiology, University of California, Irvine, CA 92717.**

UCI is an Affirmative Action/Equal Opportunity Employer

(NW3607)A

## RESEARCH ASSOCIATE/ASSISTANT PROFESSOR

A non-tenure track position for a Research Associate/Assistant Professor is available for research and teaching, with limited participation in selected course offerings. The research area is to be directed towards the molecular/cell biology of microtubules and associated proteins and their structural-functional correlation, using a broad range of techniques, including protein purification/characterization, hybridoma technology, immunochemistry and immunomicroscopy. A Ph.D or foreign equivalent and two years of postdoctoral experience are required. The position will be filled on the basis of demonstrated research experience.

Send curriculum vitae and three letters of reference by May 15, 1989 to: **Becca Vance, Department of Cell Biology & Neuroanatomy, University of Minnesota, 4-135 Jackson Hall, 321 Church St. S.E., Minneapolis, MN 55455.**

*The University of Minnesota is an equal opportunity educator and employer and specifically encourages applications from women and minorities.*

(NW3593)A



## Cancer Research Campaign

**Christie Hospital and Holt Radium Institute**

**PATERSON INSTITUTE FOR CANCER RESEARCH**

**Department of Carcinogenesis**

### POSTDOCTORAL SCIENTIST

Available immediately in an active group investigating the molecular basis of the biological effects of xenobiotic agents, principally environmental carcinogens and chemotherapeutic alkylating agents.

The successful candidate would mainly be involved in the isolation and expression of pro- and eukaryotic DNA repair genes. Experience in cell biology/recombinant DNA methodology, especially gene cloning and transfer techniques would be an advantage. Informal enquiries to Dr Geoff Margison, 061 445 8123 ext 413.

The post is available for 5 years initially under MRC conditions of employment and remuneration, depending upon age and experience.

**Please send CV and an outline of research interests and the names of 2 academic referees to Dr P J O'Connor, Head, Carcinogenesis Department, Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX, UK. (8983)A**

## THE AUSTRALIAN NATIONAL UNIVERSITY

**CENTRE FOR INFORMATION  
SCIENCE RESEARCH**

### POSTDOCTORAL FELLOW/ RESEARCH FELLOW

**(COMPUTATION USING  
NEURONAL NETWORKS)**

A position is available for an applicant with a PhD in engineering or science to investigate the use of self-organising neuronal-like networks to 1) extract features from poorly defined data, and 2) assist in understanding the integrative action of real nervous systems. Applicants should have a strong background in mathematics and computation and an enthusiasm for investigating uses of parallel processing with adjustable connectivity. No detailed knowledge of the nervous system is required. The successful applicant will work with Professor S. Redman in the Experimental Neurology Group, Division of Neuroscience, John Curtin School of Medical Research. Enquiries to Professor Redman: (062) 492602.

Closing date: 9 June 1989. Ref: IS 30.3.1.

**SALARY:** Research Fellow; A\$30,737-A\$40,100 p.a.; Post-doctoral Fellow Grade 1 (fixed point); A\$26,617-A\$30,360 p.a. **APPOINTMENT:** Research Fellow up to three years, possibility of extension to five years; Postdoctoral Fellow normally two years, possibility of extension to three years. **APPLICATIONS** should be submitted, in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from Appointments (36238), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

**THE UNIVERSITY IS AN  
EQUAL OPPORTUNITY EMPLOYER** (W6045)A

## UNIVERSITY OF BIRMINGHAM CANCER RESEARCH CAMPAIGN LABORATORIES

A post-doctoral Research Fellow required to join a collaborative project studying the structure and function of the *ras* p21 oncogene product. Protein biochemistry and nuclear magnetic resonance (NMR) investigations are underway into the mode of action of p21 and it is envisaged that the successful applicant will be able to contribute expertise in protein chemistry and/or molecular biology, cell biology or related areas of knowledge are invited to apply. Knowledge of NMR is not required. Post is for three years funded by the Cancer Research Campaign and is available immediately. Salary will be on scale £9865 - £15720 with superannuation.

Informal enquiries to Dr. Roger Grand 021-414-4481. Application forms and further particulars available from **Senior Assistant Registrar, Medical School, Birmingham, B15 2TJ, 021-414-4050 to whom applications (3 copies) should be sent by 28th April. Quote Ref. RF/CS/RG.**

**AN EQUAL OPPORTUNITIES EMPLOYER** (8949)A



## PLANT BREEDING INTERNATIONAL WHEAT BREEDERS

PBI Cambridge was established in 1988 as a Unilever Company following the acquisition, from the UK government, of the facilities and major breeding programmes of the former Plant Breeding Institute, and the National Seed Development Organisation. The wheat breeding programme has an established record of excellence and PBI varieties occupy 70% of the UK acreage.

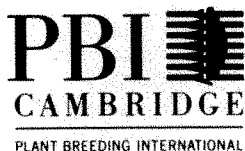
We are now seeking to appoint additional wheat breeders to strengthen the programme and extend it into Europe. The expansion is beginning with the establishment of a programme in France.

Applicants should have a good honours degree in plant science. Relevant postgraduate experience would be a distinct advantage. However, consideration will also be given to those who can demonstrate a commitment to plant breeding.

Salary will be negotiable depending on experience and qualifications.

For further particulars and application form, telephone Cambridge 840411 ext 307, or write to the **Personnel Officer, Plant Breeding International Cambridge Ltd, Maris Lane, Trumpington, Cambridge CB2 2LQ**, quoting ref WT/57.

An Equal Opportunities Employer.



PLANT BREEDING INTERNATIONAL

(8957)A

## MEDICAL RESEARCH COUNCIL TOXICOLOGY UNIT

### RESEARCH OFFICER

Required to work in a Toxicology Laboratory, investigating cell specific mechanisms of toxicity in the kidney. The position would suit a biochemist with cell culture and separation expertise. Experience in immunology and immunocytochemistry would be an advantage.

Applicants should hold a Degree or equivalent qualification in a biological science. Salary is on an incremental scale £7,443-£13,545 including London Weighting. (Pay award pending.)

For further details and application form, please contact **Dr K. Cain, Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF**. Tel 01-643 8000.

Closing date for application is 11th May 1989.

The Council is an Equal Opportunity Employer.



(9010)A

## POSTDOCTORAL POSITION

available immediately for a biophysical chemist to study cation-DNA interactions by multinuclear NMR. Send résumé and two letters of reference to **Dr. W. Braunlin, Polytechnic University, 333 Jay Street, Brooklyn, NY 11201**. Telephone 718-260-3552.

(NW3591)A

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## CEREBROVASCULAR RESEARCH SCIENTIST

to join existing faculty in the study of the development, plasticity and pathology of neurovascular function. The successful candidate will exhibit evidence of independent research productivity and the ability to attract external funding. Applicants should have experience with pharmacological, physiological and functional methods to complement present anatomical, biochemical and immunohistochemical techniques. Primary tenure-track appointment will be in the Department of Neurosurgery at the assistant or associate professor level. Please send C.V. and have three letters of recommendation forwarded to **Keith A. Crutcher, Ph.D., Research Division, Dept. of Neurosurgery, Univ. of Cincinnati, Cincinnati, OH 45267**.

(NW3604)A

*The Polytechnic operates a range of degree and post graduate level courses, has a vigorous and successful record of consultancy and research. Due to Corporate Status the Polytechnic has entered a new and challenging phase of its development. To maintain and extend the programme of work, applicants are invited for the following post:-*

## FACULTY OF SCIENCE – SCHOOL OF PHARMACOLOGY

### Head of School

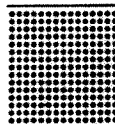
£24,084 – £26,538

Applicants for the post should possess high academic credibility and also have the ability to manage the School as a budget centre, be able to lead in academic development and entrepreneurial initiatives. The person appointed would be eligible for consideration for appointment to the Professoriate.

The School of Pharmacology is one of 3 schools in the Faculty of Science and has an establishment of 16 academic staff. The School embraces the disciplines of pharmacology and physiology.

**Applications forms and further particulars can be obtained from Personnel Services, Sunderland Polytechnic, Langham Tower, Ryhope Road, Sunderland SR2 7EE, or telephone 091 5152429.**  
Closing date: 5th May, 1989.

(9009)A



**SUNDERLAND  
POLYTECHNIC**

## ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

### DEPARTMENT OF MEDICINE — ENDOCRINE UNIT NON-CLINICAL LECTURER

Applications are invited for a lectureship in the Endocrine Unit of the Department of Medicine, Royal Postgraduate Medical School. This is a busy laboratory of about 20 scientists working in the field of regulatory peptides. Particular interests include the expression, role and pathophysiology of novel peptides in the hypothalamus, pituitary and islets of Langerhans. The successful applicant will have a PhD and be expected to contribute collaboratively to the original research of the unit. The candidate's own field of expertise and current interest is not critical. The post is for 5 years. Initial salary will be at an appropriate point on the salary scale for non-clinical Lecturers (A or B). Informal enquiries to Professor S R Bloom on 01-740 3242.

Applications in the form of a c.v. (Six copies) with the names and addresses of 3 referees should be sent to the **Personnel Officer, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN**, from whom further particulars are available (telephone 01-740 3204). The closing date for the receipt of applications is 11 May 1989. Ref: AM88

(9002)A

## RESEARCH ASSOCIATE (POSTDOCTORAL)

available immediately to study the neuronal mechanisms underlying the behavioral effects of hallucinogens, CNS stimulants and related compounds. The applicant should have a Ph.D. (or ABD) in experimental psychology, pharmacology or related biomedical science, knowledge of drug discrimination methods, and an interest in more "molecular" techniques (neurotoxin lesion, in vivo dialysis, etc.) in conjunction with this procedure. Expertise in computer programming and interfacing is desirable but not mandatory. Send curriculum vitae and three letters of reference to: **James B. Appel, Ph.D., Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208**. The University of South Carolina is an Equal Opportunity/Affirmative Action Employer.

(NW3603)A



## Inflammation, Osteoarthritis, Enzymology Research

### Team Leaders

CIBA-GEIGY Pharmaceuticals, an industry leader in inflammation research and therapy, offers outstanding career opportunities for innovative scientists interested in Inflammation, Osteoarthritis, and Enzymology.

#### Director, Inflammation Pharmacology

Lead a team of 15 Immunopharmacologists and Cell Biologists to discover anti-inflammatory agents and agents to treat autoimmune diseases and osteoarthritis. A PhD in Pharmacology, Immunology, or Biochemistry plus 8-10 years of postgraduate research in immunopharmacology is required; extensive background in humoral and cellular immunology is essential. Please respond to Dept 357.

#### Director, Enzymology and Molecular Biology

Lead a team of 20 Enzymologists and Molecular Biologists to discover agents to treat osteoarthritis, and inflammatory and cardiovascular diseases. A PhD in Biochemistry, Molecular Biology or Pharmacology plus 8-10 years of postgraduate experience in enzymology and/or molecular biology with emphasis on inflammation or cardiovascular research is required. Please respond to Dept 343.

#### Manager, Osteoarthritis Research

Lead a team of 12 Pharmacologists to develop and evaluate in vivo models of osteoarthritis, rheumatoid arthritis and other inflammatory diseases. A PhD in pharmacology with at least 5 years of postgraduate experience in the pharmaceutical industry studying inflammatory diseases is required. Please respond to Dept 355.

**These positions further require experience in initiating and implementing drug discovery projects, the ability to lead multidisciplinary research teams, and excellent communication skills.**

We offer excellent salaries and benefits, plus a high level of professional visibility and career potential. Reply in confidence, by sending your resume and salary history, indicating department code to: **CIBA-GEIGY Corporation, Pharmaceuticals Division, 556 Morris Avenue, Summit, New Jersey 07901.** We Are An Equal Opportunity Employer M/F/H/V.

Science Serving Mankind

# CIBA-GEIGY

(NW3606)A

**GBF Gesellschaft für Biotechnologische Forschung mbH,** Braunschweig, FR Germany — a government supported Research Institute of Biotechnology — offers a

### GROUP LEADER POSITION FOR CELL BIOLOGY

in the Section of Cell Biology and Genetics

The group will include in addition to the advertised position two scientists and three technicians and excellent research conditions in a new laboratory. The successful applicant will have a background in cell biology, immune biology, inflammation or bone cell differentiation and several years' post-doctoral experience. An appreciation for the possibilities of cooperation and interaction with other departments is expected.

Applications should be addressed (within six weeks of the appearance of this advertisement) to the **Personnel Dept., GBF, Mascheroder Weg 1, D-3300 Braunschweig, FRG (ref. no. 48/89).** Please include a complete c.v., list of publications, details of research activities to date. Further information can be obtained from the Section Head, Prof. Dr. John Collins (Tel. 531/6181-200). (W6050)A

## Ph.D.'s

Biotech Research Labs in Rockville, has openings for 2 Ph.D.'s.

**HIV RESEARCH:** Individual will be responsible for HIV research. We are looking for an individual who enjoys a challenge to help solve the mysteries of HIV.

**MOLECULAR BIOLOGIST:** Individual must have experience with recombinant DNA techniques. Familiarity with characterization of genomic DNA in construction of expression vectors is preferred.

We offer competitive salaries and an excellent benefits package. If interested, please submit a CV to:

**BIOTECH RESEARCH LABS**

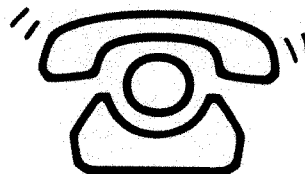
1600 East Guide Drive, Rockville, Maryland 20850. Attn: Personnel

EOE M/F/H/V

(NW3602)A

# ATTENTION

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## nature

# PFIZER ACADEMIC TRAVEL AWARDS

Pfizer Central Research at Sandwich, Kent plan to institute a new Travel Award Scheme to assist selected academic staff at British Universities, or equivalent institutions, to attend national or international symposia relevant to their field of research. Up to ten Awards will be made annually by Pfizer, on the basis of academic research achievement and with a bias towards younger applicants.

The Awards will each be up to £500 in value. Five will be allotted to academic staff in U.K. departments of Organic Chemistry and five to departments of Biological Sciences.

Application forms can be obtained by writing to Dr. P. R. Leeming, Pfizer Central Research, Sandwich, Kent CT13 9NJ. The closing date for application

for 1989-1990 Awards is 31st May and will be available for symposia commencing 1st July, 1989-30th June, 1990.

These travel Awards are the latest in a series of initiatives taken by Pfizer to support academic research in the U.K., and in particular to help young scientists early in their careers.

Pfizer is a worldwide, research-based company with businesses in health care, agricultural, specialty chemicals, materials science and consumer products. The Company has a major commitment at Sandwich where it employs approximately 1,700 people, of whom nearly 700 are engaged in new product research.



(8948)N

## PEARSE PRIZE AND LECTURESHIP

To honour the contributions made to histochemistry by Professor A.G.E. Pearse a prize was established which is administered by the Royal Microscopical Society. The prize should be seen to be one of the major international honours in Histochemistry and will be awarded approximately every two years. It is envisaged that it will be given to a scientist who has contributed significantly to Histochemistry and is still active.

Nominations are now called for the 1990 PEARSE PRIZE to be awarded to a scientist who has made a valuable contribution either

- (a) to the development of histochemical techniques
- or (b) to the application of histochemical methods.

The name of each candidate should be accompanied by

- 1) a letter of recommendation containing a statement of reasons for the nomination, signed by two people who are familiar with the candidate's work,
- 2) a list of publications of the candidate,
- 3) the written consent of the candidate.

The Prize winner will receive a medal and be invited to deliver a lecture to the Royal Microscopical Society.

Nominations should be sent to the **Pearse Prize Committee, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, U.K.** to be received by 30th November 1989. (8993)N

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## FELLOWSHIPS

### MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

#### FELLOWSHIP IN MOLECULAR BIOLOGY

The Council of the Association wishes to promote the application of molecular biological techniques to fundamental problems that can be best approached by exploiting the diverse range of species available in the sea. We are particularly interested in problems associated with the evolution of the vertebrates and embryonic development.

Applications are therefore invited for a five year post-doctoral Fellowship, to be held at the Association's Laboratory in Plymouth where there are good facilities for catching and maintaining a wide variety of marine species. The successful candidate will be encouraged to spend some time in the laboratory of Dr S Brenner (MRC Laboratory of Molecular Biology, Cambridge) acquiring the appropriate techniques. MBA Fellows are also encouraged to have direct links with University Departments.

The MBA Laboratory is housed at Citadel Hill together with components of the Plymouth Marine Laboratory (PML) of NERC and relevant research underway includes the molecular aspects of the response of marine organisms to environmental stimuli, cell signalling mechanisms during development, and the molecular genetics of *Sagitta*. There is also an active Visitors Programme with a strong emphasis on cephalopod neurobiology and molecular and physiological aspects of the development of *Ciona*. We are particularly interested in applicants whose project can relate to this on-going work.

Application should be made to **Dr M Whitfield, Director, Marine Biological Association, Citadel Hill, Plymouth, PL1 2PB**, from whom further information on the Association's Research Programme may be obtained. Applicants should include a statement indicating their proposed area of research, a CV and the names of three referees. The closing date for applications is 30 May 1989. Salary will be assessed by analogy with Civil Service scientific scales according to age, qualifications and experience. The starting date will be by arrangement. (8950)E



# WOOD/WHELAN INTERNATIONAL UNION OF BIOCHEMISTRY (IUB) AND INTERNATIONAL COUNCIL OF SCIENTIFIC UNIONS (ICSU) RESEARCH FELLOWSHIPS

## Objectives

The Wood/Whelan IUB and ICSU Research Fellowship are designed to support biochemists who need to travel to other laboratories in the IUB/ICSU areas for the purpose of carrying out experiments requiring special techniques or for other forms of scientific collaboration or advanced training.

## Conditions of the Fellowships

The fellowships will be awarded for short periods (2 weeks to 2 months, exceptionally 3 months). The fellowships will cover travel costs on the basis of economy or tourist fares (coverage will only be partial for long distances). A basic subsistence allowance of US\$25/day will be allotted to fellowship holders, with a geographical adaption factor. There will be no additional stipend for dependents and no provisions made for accidental or health insurance, which are expected to be contracted privately by the fellows.

Recipients of the fellowships are required to make a full declaration to IUB/ICSU of all other support received towards the same travel and subsistence. IUB/ICSU may reduce its financial contribution accordingly. IUB/ICSU fellowships cannot be used to supplement other full time support fellowships.

## Applications

Applications should be sent in triplicate with the following documents:

- A research proposal of about two pages typescript indicating clearly: (1) the nature of the project and the type of experiments to be carried out; (2) why it is necessary to travel to another laboratory to conduct the experiments rather than to perform them in the applicant's own laboratory or simply ship the materials; (3) why the particular laboratory has been selected; (4) why the project will require the particular time period requested. If the aforementioned material is not sufficiently clear, the application is likely to be rejected or the decision on it seriously delayed.
- A short curriculum vitae of the applicant with a list of publications indicating names and other authors
- A letter of acceptance from the head of the receiving institute and signed by the leader of the group which will receive the recipient.
- A letter of recommendation from the head of the department of the applicant's institution indicating support of the applicant and the reasons why the fellowship would be beneficial. This letter should also list all other fellowships previously received by the applicant, especially for travel abroad to attend meetings or study at another institution.

**Applications from Africa, Europe or North America should be sent to Dr. Marianne Grunberg-Manago, Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, 13, rue Pierre et Marie Curie, 75005 Paris, France.**

**Applications from Latin America or Asia should be sent to Dr. Jorge E. Allende, Departamento de Bioquímica, Facultad de Medicina (Norte), Universidad de Chile, Casilla 6671, Santiago 7, Chile.**

Applications can be submitted at any time but they will be reviewed twice each year, in June and December.

## Criteria for Selection of Fellows

The criteria for the selection of applicants are:

- Excellence of qualifications of the applicant.
- Need to travel to do the experiments and availability of other sources of funds to finance travel. Only exceptionally will support be given to senior scientists or to heads of departments. Fellowships will not be awarded to attend courses, symposia, meetings or congresses.
- Geographical distribution.

(NW3594)E

**ROCHE**

## Postdoctoral Fellowships in Molecular Biology/ Mammalian Development

Positions are available in the Department of Dermatology and Oncology for postdoctoral fellows to study the effects of retinoids on mammalian embryonic development using molecular biological approaches. Experience in one or more of the following areas is desirable: embryology, cell biology, or molecular biology.

The positions will be initially for one year, with extensions foreseen for an additional year.

Interested individuals are invited to send a copy of their c.v., including a list of publications, and letters from three referees to the **Personnel Department**, quoting reference Nature 73/89/WA.

F. Hoffman-La Roche & Co. Limited Company,  
CH-4002 Basle (W6042)E

**INSTITUTE OF CANCER RESEARCH**  
(University of London)  
Fulham Road, London

## POSTDOCTORAL FELLOWSHIP

A postdoctoral fellowship is available for a molecular biologist/biochemist to join a research team (lead by Dr. G. Goodwin) within the Department of Cell and Molecular Biology at the Chester Beatty Laboratories. The project is concerned with the molecular cloning of three transcription factors which we have identified binding to regulatory elements of the c-myc proto-oncogene.

Experience of molecular biology techniques is desirable.

The position is funded for three years and salary is in the range of £13130 to £15950 inclusive.

Informal enquiries can be made by contacting Dr. G. Goodwin on (01) 352 8133.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

Please send applications in duplicate with names and addresses of two referees to the **Personnel Officer**, 17A Onslow Gardens, London SW7 3AL quoting reference 4.89.S.N.2 (8954)E

## Skin Cancer Research Post Doctoral Fellowship University of Alberta

Two positions immediately available to study UV-photo-carcinogenesis and immunobiology of malignant melanoma. The amount awarded will be equivalent to MRC and NRC guidelines, with future opportunities to apply for independent research and faculty positions.

Send a resume and three references to: **Kowichi Jimbow, M.D., Ph.D., Professor and Director, Division of Dermatology and Cutaneous Sciences, 418 Newton Research Building, University of Alberta, Edmonton, Alberta, Canada T6G 2C2.**

The University of Alberta is an equal opportunity employer  
(NW3596)E

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**MEDICAL RESEARCH COUNCIL**  
LABORATORY OF MOLECULAR BIOLOGY  
Hills Road, Cambridge, CB2 2QH

**Postdoctoral Fellowship**

Applications are invited for a postdoctoral fellowship (sponsored by Applied Biosystems Ltd) to work in one of the following areas:

- 1) DNA sequencing methodology and application to large genomes. Development of computer programmes to analyse DNA sequences (Bart Barrell).
- 2) Synthesis of oligoribonucleotides and studies of ribozymes and RNA-protein interactions (Michael Gait).
- 3) Development of protein microsequencing methods and their application to mammalian complex I and proteins involved in neurodegenerative disease (John Walker).

The position is available immediately and is for 3 years. Salary in the range £11070-£19,310 per annum (under review).

Applications should include a full curriculum vitae and the names, addresses and telephone numbers of at least 2 referees and should be sent by 8 May 1989,

quoting reference ABL to:

**The Personnel Officer**  
**MRC Centre**  
**Hills Road**  
**Cambridge**  
**CB2 2QH**

**MRC**  
Medical Research Council

*The Medical Research Council is an Equal Opportunity Employer.*  
(8977)E

**POSTDOCTORAL FELLOWSHIP**

NIH Staff Fellow/Senior Staff Fellow position is available at the National Institute on Alcohol Abuse and Alcoholism, for studies of neurotransmitter and hormone receptors and their interactions with cytokines and growth factors. Preference is given to applicants with experience in molecular biology, protein purification and receptor biochemistry/pharmacology. Salary is commensurate with experience. Only U.S. citizens or permanent residents are eligible for this position.

Send CV and name of three references to:

**Dr. George Kunos**  
**Laboratory of Physiologic and**  
**Pharmacologic Studies NIAAA**  
**12501 Washington Avenue**  
**Rockville, MD 20852**  
**Phone: (301) 443-1234**



NIAAA IS AN EQUAL OPPORTUNITY EMPLOYER

(NW3561)E

**BIOZENTRUM — UNIVERSITÄT BASEL**  
**BIOCENRE-FELLOWS**

Applications are invited for an **independent Postdoctoral Research Position**. Candidates with a recent PhD or with postdoctoral experience should not be more than 33 years of age. Fellows are offered a contract of three years including salary and running costs. The position is available immediately or upon agreement; it is neither renewable nor tenure-track.

Biocentre-Fellows will have complete scientific independence, will be able to work in the department of their choice and will have access to all shared equipment as well as the technical and administrative services of the Biocentre. They may engage in teaching activities but are not obliged to, and they may apply for additional research funds elsewhere.

Biocentre-Fellows will be chosen by a special committee which will provide additional detailed information upon written request. Candidates should submit a curriculum vitae, a list of publications, a brief description of the proposed research (not more than 3 pages), and the names of three references, to

**Dr. Ch. Brack**  
**Biozentrum der Universität Basel**  
**Klingelbergstr. 70**  
**CH-4056 Basel**  
**Switzerland**

(W6053)E

**STUDENTSHIPS**



*University*  
*of Reading*  
**Appointments**

**DEPARTMENT OF MICROBIOLOGY**

**MRC RESEARCH STUDENTSHIP**

Applications are invited for an MRC Research Studentship tenable for 3 years, commencing October 1989, to work towards a Ph.D. degree with Dr. M. J. C. Crabbe on novel antibiotic and antiviral compounds. The project will focus on molecular enzymology and molecular modelling of biosynthetic intermediates and enzymes. It will involve the use of techniques in protein chemistry, computer modelling and recombinant DNA. There will be ample opportunity for interactions with research groups in the Departments of Microbiology and Chemistry. Candidates should have, or expect to obtain, a first or upper second class honours degree in the biochemical- or chemical-related sciences.

Applications, including a CV and the names and addresses of two referees, should be sent to **Dr. M. J. C. Crabbe, Reader in Microbiology, London Road, Reading RG1 5AQ (Tel: 0734 318894; FAX: 0734 750140; Email: SKSCRABB@UK.AC.RDG.AM.CMS)**, from whom further details may be obtained.

(8989)F

**UNIVERSITY OF ABERDEEN**

**Department of Genetics and Microbiology**

**NERC Research Studentships**

are available on the following topics:

The role of soil animals in microbial dispersal, survival, growth and gene transfer (with Depts. of Biochemistry and Plant & Soil Science and NERC ITE, Merlewood).

Gene transfer in biofilms (with Dept. of Biochemistry)

Urease production and autotrophic nitrification in acid soils  
Zoospore taxis and the electrochemical nature of the rhizosphere  
Chitin deacetylation — ecological and biotechnological significance

Studentships will also be available in the following areas:

Molecular biology of membrane transport systems  
Thermophilic sulphate reducing bacteria  
Site directed mutagenesis  
Genetics of ovarian cancer.

Applications should be sent as soon as possible to **Professor W. A. Hamilton, Department of Genetics and Microbiology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS. (Tel. 0224 273144).**

(8979)F

**OXFORD UNIVERSITY**

**Nuffield Department of Surgery**

**RESEARCH STUDENTSHIP**

Applications are invited for an MRC Research Studentship leading to the degree of DPhil under the supervision of one of the following: Dr. J. Austyn (maturation and migration of dendritic leucocytes); Dr. M. Dallman (the role of lymphokines in graft rejection); Dr. S. V. Fuggle (in situ hybridisation of molecules involved in cellular interactions); Dr. D. R. Gray (pancreatic islet purification by cell sorting); and Dr. K. J. Wood (mapping of active amino acids involved in the induction of allogenic unresponsiveness).

The studentship is tenable for 3 years from October 1989. Applicants should have, or expect to obtain, a First or Upper Second Class Honours degree in one of the Biological Sciences.

Applications, including a CV and the names and addresses of two academic referees should be sent to **Professor P. J. Morris, Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU by May 15th, 1989.** (8943)F

DEPARTMENT OF ZOOLOGY  
UNIVERSITY OF ABERDEEN

## PhD RESEARCH STUDENTSHIPS

### 4 awards from SERC and 4 awards from NERC

Applications are invited from undergraduates who expect to graduate this year or from students already graduated with a good honours degree in the appropriate subject for the following vacancies.

**SERC** (Quota and CASE) to be selected from:

- 1 Invertebrate neuroendocrinology (Prof Mordue)
- 2 Arthropod neurophysiology — interneurons (Dr Fraser)
- 3 Olfactory communication in mammals (Dr Gorman)
- 4 Animal nutrition, energetics and growth (Dr Houlihan)
- 5 Fish immunology (Dr Secombes)
- 6 Insect-plant relationships: the role of plant secondary compounds (Dr Mordue)
- 7 Parasite chemotherapy and reproductive biology (Dr Chappell)
- 8 Energetics of predatory fish (Dr Friede)

**NERC:**

- 1 Pheromones in wood ant foraging (Drs Ollason & Young)
- 2 Land use and herbivore feeding strategies (Professor Racey, CASE with Macaulay Institute and Forestry Commission)
- 3 Parasite infection strategies in fish and molluscs (Dr Chappell)
- 4 Effects of omnivores and parasites on food webs (Drs Raffaelli & Pike)

Applicants should write to the Head of Department or make direct contact with the staff member concerned.

Address: **Department of Zoology, University of Aberdeen, Tillydrone Avenue, ABERDEEN, AB9 2TN**

Tel: 0224-272858

DEP 4/1.28

(8981)F

## UNIVERSITY OF LEEDS

### DEPARTMENT OF BIOPHYSICS

#### SERC MOLECULAR RECOGNITION CENTRE RESEARCH STUDENTSHIPS 1989

for graduates in Chemistry, Physics, Biochemistry, Biophysics or Computer Science.

Several SERC and MRC Research Studentships are available for projects including the following topics:

Copper quinoproteins: sequencing and mutagenesis of the galactose oxidase gene.  
Structural studies of bacterial Arg and Met repressors and their interactions with synthetic operators.

Structural studies on the mechanism of inhibitors of dihydrofolate reductase.

Modelling the tar stem-loop region of the RNA of human immunodeficiency virus (the "Aids" virus).

Ligand-binding and stabilization of beta-lactoglobulin.

(CASE Studentship with Hannah Research Institute)

X-ray and optical microscope studies of liquid crystalline phases of anti-asthmatic and anti-allergic drugs, dyes and nucleic acids.

Synthesis and structural studies of sulphated carbohydrates.

(CASE Studentship with AFRC Institute for Food Research)

The Biophysics department is excellently equipped for the study of molecular structure and functions by X-ray crystallography, computer graphics and spectroscopy; extensive collaboration with the Biochemistry, Genetics and Biotechnology groups in Leeds provides an exciting research environment.

Candidates must expect to obtain a good honours degree in a relevant subject and should apply to:

**Professor A.C.T. North, Department of Biophysics,**

**University of Leeds, Leeds LS2 9JT (telephone 0532 333022) (8978)F**



## HANNAH RESEARCH INSTITUTE

### AFRC POSTGRADUATE STUDENTSHIPS

Two AFRC studentships are available from 1st October 1989 to study:

(1) Adipogenic factors: this involves isolation, characterisation and immunological manipulation of factors which promote the development of adipose tissue in farm animals, and is supervised by Drs Vernon, Flint and Clegg.

(2) Autocrine mechanisms

modulating endocrine control of mammary gland function: this involves investigation of the control of receptors for hormones and growth factors in the mammary gland by local mechanisms sensitive to milking frequency or efficiency, and is supervised by Dr Wilde.

Further information can be obtained by contacting the supervisors (telephone 0292 76013). Letters of application accompanied by a CV (including names and addresses of two academic referees) should be sent to: The Secretary, Hannah Research Institute, Ayr, KA6 5HL. (8975)F

## THE UNIVERSITY OF NEWCASTLE UPON TYNE GENETICS

### SERC RESEARCH STUDENTSHIPS 1989

Two SERC studentships are available within the Department starting on 1 October 1989, to work on (1). Cryoenzymology of Penicillin acylase from *Escherichia coli*, and (2). Domain structure and function with the pentafunctional AROM polypeptide of *Aspergillus nidulans*. The projects will involve low temperature solution techniques to study catalytic mechanisms and intermediates, and the use of the powerful techniques of molecular biology to isolate and characterise functional protein domains and synthesise proteins with unique enzymatic properties.

Candidates should have or expect to obtain a 2(i) degree (or equivalent) in a relevant subject, and are encouraged to phone Richard Virden (091-2227432) or Alastair Hawkins (091-2227673) to discuss the projects in more detail. Applications should contain a full curriculum vitae and the names and addresses of two academic referees and be sent to the **Department of Biochemistry and Genetics for the attention of Dr. Richard Virden (project 1) or Dr. Alastair Hawkins (project 2) as soon as possible.** (8991)F

## UNIVERSITY OF BIRMINGHAM DEPARTMENT OF IMMUNOLOGY

### Ph.D Research Studentship

Applications are invited from candidates wishing to pursue a full-time three-year Ph.D programme of investigation into biochemical and biological aspects of B lymphocyte derived growth factors. This is an MRC funded project which will be under the supervision of Dr John Gordon and his team, with the work being carried out in a multi-disciplinary environment.

Written applications including a C.V. and the names and addresses of two referees should be sent as soon as possible to **Dr John Gordon, Department of Immunology, West Extension, The Medical School, Vincent Drive, Edgbaston, Birmingham, B15 2TJ.**

Informal enquiries to Dr Gordon (021.414.4034).

(8969)F

## UNIVERSITY OF SOUTHAMPTON CLINICAL PHARMACOLOGY GROUP AND MEDICINE RESEARCH STUDENTSHIP

Applications are invited for a three year research studentship to commence in October 1989 researching into inflammatory factors causing human gastrointestinal diseases and drugs used in their treatment. The Group has excellent equipment and well funded projects. The student will receive broad training suitable for an industrial or academic career in a unit committed to high quality collaborative research. The University and surrounding area have excellent recreational facilities.

Applicants should hold or expect to obtain a good degree in the biological sciences.

Please send a full curriculum vitae and the names and addresses of two referees as soon as possible to **Dr K. Hillier, Clinical Pharmacology Group, Medical & Biological Sciences Building, Bassett Crescent East, Southampton SO9 3TU,** from whom further details can be obtained by telephoning 0703-595000, ext. 4264, or writing.

(8922)F

## FELLOWSHIPS CONTINUED FROM PAGE 21

### University of Western Ontario

#### Post Doctoral Fellowship in Applied Mathematics

Applications are invited from candidates possessing a Ph.D. in a suitable branch of pure or applied mathematics to carry out research on integral transforms occurring in wave propagation problems. Stipend \$1650-\$1950 per month.

Applications naming two referees should be sent to **Dr. D. Naylor, Department of Applied Mathematics, University of Western Ontario, London, Canada, N6A 5B9** (NW3597)E

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## STUDENTSHIPS

### UNIVERSITY OF OXFORD



#### NEUROSCIENCES GROUP INSTITUTE OF MOLECULAR MEDICINE JOHN RADCLIFFE HOSPITAL

Applications for the following posts are invited from final year students expecting to get a good honours degree, to work in a multi-disciplinary research group studying the human autoimmune disease myasthenia gravis.

#### MRC RESEARCH STUDENTSHIPS

£3,125 per annum (allowances available for mature students and for those with previous experience) for a period of three years.

The student would work on one of the following projects:

- (1) Eukaryotic expression of human acetylcholine receptor
- (2) Presentation of recombinant human acetylcholine receptor to human T cell clones
- (3) Expression of acetylcholine receptor epitopes and mRNA in human thymus and thymoma

#### RESEARCH ASSISTANT — Grade 1B

Salary: £8,675-£13,265.

To investigate the effects of myasthenic antibodies on ion channels expressed by neuronal cell lines, using voltage/patch clamp recording techniques. This post is funded by Action Research for a period of three years.

Applications, including a cv and the names of two referees, should be sent to **Professor J. Newsom-Davis, Neurosciences Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU**. Further details can be obtained by telephoning (0865) 752322. (9004)F

*The University is an Equal Opportunity Employer*

#### UNIVERSITY OF BRISTOL SCHOOL OF CHEMISTRY ORGANIC GEOCHEMISTRY UNIT NERC RESEARCH STUDENTSHIPS

Opportunities exist for research leading towards a Ph.D. degree in the following areas:-

1. **Bacterially mediated sulphur incorporation into organic matter in marine sediments** (CASE award with the Scottish Marine Biological Association, Oban). Recent studies have revealed the presence of novel sulphur containing lipids in marine sediments. The student will investigate the pathways to, and rates of production of, such lipids, using a combination of modern chemical and microbiological methods.
2. **Biomarkers of bloom forming marine phytoplankton** (CASE award with the Scottish Marine Biological Association, Oban). Studies of the biolipid distributions of natural blooms, and cultured samples grown and biodegraded under a variety of conditions, will be used to interpret the sedimentary record.
3. **Biogeochemistry of diagenetic fronts in deep sea sediments** (CASE award with the Institute of Oceanographic Sciences, Deacon Laboratory). Sedimentary cores taken from the Atlantic show evidence of postdepositional microbial 'burn down'. The student will be part of a team investigating the associated molecular (lipid) changes with a view to better interpretation of the geological record.
4. **Chitin in the fossil record**. Chitin is a complex polysaccharide found in a range of organisms which are primary contributors of animal organic matter to sediments. The project will investigate the influence of environmental parameters on chitin degradation by sediment bacteria, including the analysis of the degradation products, and will involve field work on exceptionally preserved fossil occurrences.

Applicants should have, or expect to obtain, a good Honours Degree in Chemistry or Biochemistry (1-4), Microbiology (1,4), or Earth Sciences with a component from one of these fields (4). **Applications to Dr. J.R. Maxwell (1), Professor G. Eglinton (2-4), Organic Geochemistry Unit, University of Bristol, School of Chemistry, Cantock's Close, Bristol BS8 1TS.** (8996)F

#### IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE DEPARTMENT OF PURE & APPLIED BIOLOGY POSTGRADUATE STUDENTSHIPS

Studentships are available in the Department of Pure and Applied Biology. Applications will be welcomed for the following projects:

- |   |   |
|---|---|
| <p>(a) Immuno-epidemiology of gastro intestinal nematode infections.</p> <p>(c) Behaviour of aphids in relation to virus transmission and treatment</p> <p>(e) Electrophysiological and structural studies of chitin-secreting cells.</p> <p>(g) The antigenic architecture of the liver stages of malaria: a primary analysis of novel vaccine or drug targets.</p> <p>(i) The use of transgenic plants to study geminivirus DNA replication.</p> <p>(k) Biochemical and neurophysiological studies on the selective toxicity of second generation avermectins against lepidoptera.</p> <p>(m) Population dynamics of insect-parasitic nematodes.</p> <p>(o) Mechanisms of selective herbivory in foliar and root-feeding insects.</p> <p>(p) Investigating the manganese-protein complex, using EPR and biochemical techniques.</p> <p>(r) The analysis of predation and parasitism in a field population of insects.</p> <p>(t) Interactions between foliar and root-feeding insects.</p> <p>(v) Development of an image analysis system for studying arthropod behaviour.</p> <p>(x) Signals and receptors in nematode diapause.</p> <p>(aaa) Isolation and functional importance of the products of the ndh genes of the chloroplast genome.</p> | <p>(b) Transmission dynamics of infectious diseases.</p> <p>(d) <i>In vitro</i> and <i>In vivo</i> investigation of insecticide antimicrobial drugs.</p> <p>(f) Digestive physiology of parasitic arthropods.</p> <p>(h) Molecular organisation and expression of tobacco necrosis virus.</p> <p>(j) Recombination mechanisms in fungi.</p> <p>(l) Structure-activity relationships of novel avermectins in insects.</p> <p>(n) The study of primary photochemistry of photosynthetic pigment-protein complexes using picosecond and femtosecond time time-resolved laser spectroscopy.</p> <p>(q) Effects of elevated CO<sub>2</sub> concentrations on crop/pest interactions.</p> <p>(s) The role of polyphagous parasitoids in dynamics of leaf minor communities.</p> <p>(u) Knowledge based systems for cereal aphid control.</p> <p>(w) The assessment of canopy structure in Calluna heathlands through remote sensing.</p> <p>(y) Electron transport mechanisms in nematodes.</p> <p>(z) Determinants of symptom induction in plants by red clover necrotic mosaic virus.</p> |
|---|---|

The studentships will be funded by SERC, NERC, MRC and AFRC. Applicants should have or expect to obtain a 2.1 or 1st class honours degree in a Biological Science subject.

Apply by submitting a curriculum vitae, including the names of two academic referees to the **Academic Administrator, Department of Pure & Applied Biology, Imperial College, Prince Consort Road, London SW7 2BB**, or telephone 01-589 5111 extn 7407 for further details. (9008)F



### UNIVERSITY OF ABERDEEN

#### Advance Course Studentships M.Sc. in Human Metabolism and Nutrition (Research and Experimental Methods)

Applications are invited for Research Council Advance Course Studentships for this full time 12 month course which provides systematic instruction and practical experience in the fundamental methods and techniques relevant to human metabolism and nutrition in health and disease. The course is designed for graduates who wish to pursue a career in which a critical and innovative approach to human nutrition is important.

Candidates should have a medical qualification or a good honours degree in a biomedical science.

Closing date for applications is June 30th 1989.

**For further details and application forms write to Dr C E Casey, Department of Medicine & Therapeutics, University of Aberdeen, Polwarth Building, Rm 301, Foresterhill, Aberdeen AB9 2ZD.**

(8955)F

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## STUDENTSHIPS continued

**UNIVERSITY OF BRISTOL and WELLCOME FOUNDATION LTD  
MRC Collaborative Research Studentship**

A studentship is available from 1st October, 1989, to study "The mechanisms of lactate and pyruvate transport across the plasma membrane of the malarial parasite". The project will involve culturing the malarial parasite, measurement of transport kinetics, identification and purification of the carrier and production of monoclonal and polyclonal antibodies. The work will form part of a programme of research directed towards finding alternative forms of chemotherapy for malaria that has become resistant to quinine-related drugs. The project will be supervised by Dr. A.P. Halestrap in the Department of Biochemistry at the University of Bristol, and Dr. W.E. Gutteridge in the Biochemical Sciences Department of the Wellcome Research Laboratories, Beckenham, Kent. Personal remuneration of the student will be in excess of £4000 per annum, and he will be expected to spend periods of time totalling not less than a year at the Wellcome laboratories.

Applications are invited from honours Biochemistry or Microbiology students who expect to gain at least an upper second class degree and should be made to Dr. A.P. Halestrap, Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD (Tel. 0272 303030) by 12th May. A curriculum vitae and the names and addresses of 2 academic referees should be included. (8997)F

**PATERSON INSTITUTE FOR  
CANCER RESEARCH**

CHRISTIE HOSPITAL &  
HOLT RADIUM INSTITUTE

**MRC POSTGRADUATE  
RESEARCH STUDENTSHIP**

An MRC funded PhD studentship will be available in the Paterson Institute from 1st October 1989 supervised by Professor T M Dexter and Dr J Gallagher. The studentship is for 3 years and will carry the normal MRC stipend.

The purpose of the research project is to study the role of the extracellular matrix (ECM) of bone marrow in the regulation of normal haemopoiesis and in the development of leukaemia. It is anticipated that special emphasis

will be placed on the heparan sulphate proteoglycan component of the ECM and the molecular basis of its interaction with growth factors.

The Paterson Institute for Cancer Research is a multidisciplinary research centre offering excellent opportunities for research.

Applicants should have, or expect to gain, a first or upper second class honours degree in an appropriate subject. Please submit a detailed CV including the names of two academic referees to Professor T M Dexter, Paterson Institute for Cancer Research, Christie Hospital & Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, to arrive no later than 4th May 1989. (8959)F

**UNIVERSITY OF BRISTOL**
**Research Assistantship in the Department of Geology  
Homology and convergence**

A three-year post, starting in October, 1989, is available as part of a project on Homology, convergence and taxonomic characters of fossil and living tetrapods. The project is funded by SERC, and will be supervised by Dr M J Benton. The work involves study of the morphological characters, comparison of competing cladistic phylogenies using computerised techniques. Candidates should possess a good honours degree in Biology Sciences and/or Geology. This work will lead to submission for a Ph.D.

Salary on scale £8,675 – £11,680 (Research 1B scale, under review).

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the **Personnel Office, Senate House, Bristol BS8 1TH**. Please quote reference A337.

Closing date three weeks from the date of appearance of this advertisement.

An Equal Opportunities employer.

(8995)P

## COURSES &amp; CONFERENCES


**Royal Postgraduate Medical School**

(University of London)

**M.R.C. Clinical Research Centre  
Northwick Park Hospital &  
Department of Clinical Oncology,  
Royal Postgraduate Medical School**

# Viruses, Cancer and Aids

19-21st. June 1989

The first international symposium in this exciting area drawing together interested scientists and clinicians. Organised by the M.R.C. Clinical Research Centre and the Royal Postgraduate Medical School.

**Speakers include:**

W. Bodmer	London	S. Salk	N.I.H.
P. Farrell	London	J. Weber	London
R. Gallo	N.I.H.	H. Thomas	London
K. Sikora	London	D. Onions	Glasgow
B. Griffin	London	P. Volberding	San Francisco
W. Gullick	London	A. Dalglish	London
R. Honess	London	K. Vousden	London
R. Rickinson	Birmingham	D. Crawford	London
C. Rooney	London		

**Topics:**

EBV, HBV, HPV6, papillomaviruses, HTLV-1, HTLV-2, HIV, Kaposi's sarcoma, animal models of oncogenesis, tumour suppressor genes.

**Organisers:** A. G. Dalglish  
K. Sikora

**Course fee (including catering): £200**

**Further details from: Wolfson Conference Centre  
Royal Postgraduate Medical  
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Hammersmith Hospital  
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**Telephone:** 01 740 3117 (8951)M

**Conference on: TRANSGENIC MICE AND  
MUTANTS IN MHC RESEARCH**

The Jackson Laboratory, Bar Harbor, Maine  
June 24-28, 1989

Organized by Dr. Igor Egorov, The Jackson Laboratory and Dr. Chella David, Mayo Clinic. Sessions: Expression and function of class I and class II MHC genes in transgenic mice; Mutant models of MHC antigen expression and function; Regulation of MHC gene expression; Transgenic models of disease; Both mouse and human MHC will be discussed. Contact: Linda Fournier, The Jackson Laboratory, Bar Harbor, Maine, 04609, USA, Tel (207) 288-3371. (NW3581)C

## International Conferences Organised by IBC Technical Services Ltd.



**ADENOSINE AND ATP —**  
Progress in Research and Therapeutic Potential  
25th/26th September 1989 — London

**BIOSENSORS '89**  
28th/29th September 1989 — Cambridge, UK

**SIXTH EUROPEAN SEMINAR AND EXHIBITION ON  
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5th/6th October 1989 — London

**THE PLATELET IN HEALTH AND DISEASE**  
24th/25th October 1989 — London

**OSTEOPOROSIS**  
1st/2nd November 1989 — Cambridge, UK

**PROGRESS IN SCHIZOPHRENIA**  
23rd November 1989 — London

**DRUG DELIVERY AND TARGETING SYSTEMS**  
Latest Advances  
30th November/1st December 1989 — London

**MECHANISMS OF BRONCHIAL HYPERREACTIVITY AND  
DEVELOPMENT OF ANTI-ASTHMA DRUGS**  
11th/12th December 1989 — London

**CURRENT AND FUTURE PROSPECTS  
IN THE DRUG TREATMENT OF OBESITY**  
13th/14th December 1989 — London

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This Symposium is part of a series on nutritional science funded by an unrestricted nutrition research programme of the Bristol-Myers Company of the United States.

**Range of Topics Covered:** The subjects covered on the first day will include the use of stable, nonradioactive, isotopic techniques to study energy balance and caloric requirements, as well as protein, vitamin and mineral status. On the second day, topics which will be considered are the measurement of body composition using classical and modern approaches, such as neutron activation analysis, total body electrical conductivity, bioimpedance, NMR imaging, CAT scanning and photon-densitometry. There will also be opportunities for 'hands on' inspection of some of the techniques in action on the optional third day (13 September).

**Symposium speakers will include:** Prof M J Rennie (Scotland); Prof V R Young (USA); Dr P D Klein (USA); Dr H J Powers (England); Prof J R Turnland (USA); Dr D Halliday (England); Dr R G Whitehead (England); Dr W A Coward (England); Dr A M Prentice (England); Dr M Elia (England); Prof W P T James (Scotland); Prof J S Garrow (England); Prof A F Roche (USA); Dr S H Cohn (USA); Prof M Fiorotto (USA); Prof H C Lukaski (USA); Prof G B Forbes (USA); Dr E M Widdowson (England); Prof L D Hall (England); Dr A K Dixon (England); Dr A Horsman (England); Dr P Tothill (Scotland); and Prof H W Wahner (USA).

**Symposium Organisers:** Dr Roger Whitehead and Dr Ann Prentice

**Fee:** (including residence for three nights, all meals, banquet and social events): £220 incl. VAT.

**Continuing Medical Education Creditation:** Will be available via the University of Cincinnati.

**Application forms available from:**

Mrs Maureen Gammons	or: Ms Susan Yarin
MRC Dunn Nutrition Unit	Bristol-Myers Company
Downhams Lane	345 Park Avenue
Milton Road	New York
Cambridge CB4 1XJ	NY 10154
England	USA

A programme will also be organised for accompanying persons. (8964)M



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Department of Biochemistry  
University College London  
26-30 June 1989



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**Course fee: £750** (exclusive of accommodation)

Bed and breakfast accommodation can be provided in a local hall of residence at £84 for the week.

Write for application form and further details to:

H A White  
Department of Biochemistry  
University College London  
Gower Street, London WC1E 6BT

Numbers will be limited. Closing date for applications:  
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Daniel E. Koshland Jr., Ph.D.  
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Eric Lander	Tasuku Honjo	Thomas Caskey	Raymond White
Robert Moyzis	Jean Dausset	Russell Doolittle	Norman Arnheim
Charles Cantor	Hans Zachau	Cassandra Smith	Renato Dulbecco
James Watson	Ronald Davis	Peter Dervan	Michio Oishi
Victor McKusick	Peter Pearson	David Cox	

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Volume 338 No. 6218 27 April 1989 £1.95



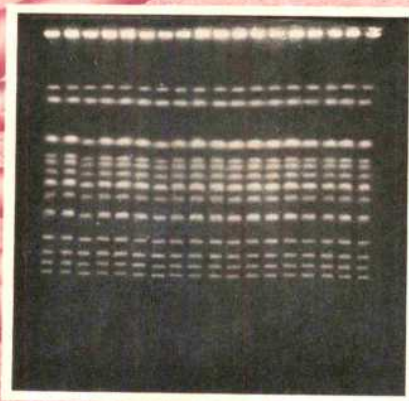
**SCIENCE IN EUROPE**

**OLD FUSION LATE**

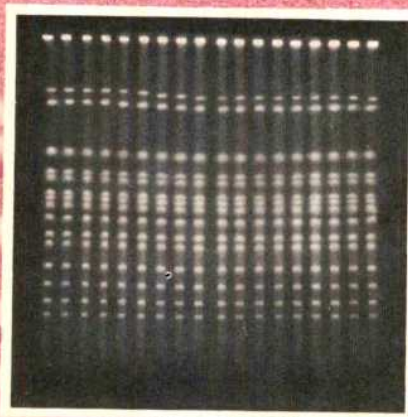


# Something you should know ...

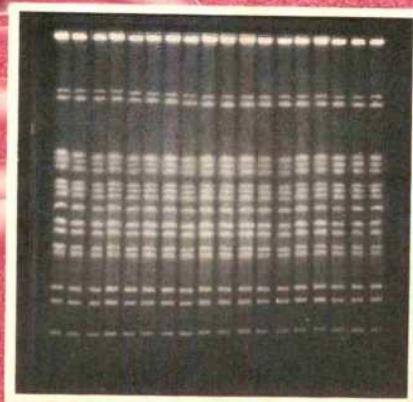
\* Taxonomy based on genomic analysis \* Chromosome isolation \* Genome mapping \* High speed DNA separation



13 bands –  
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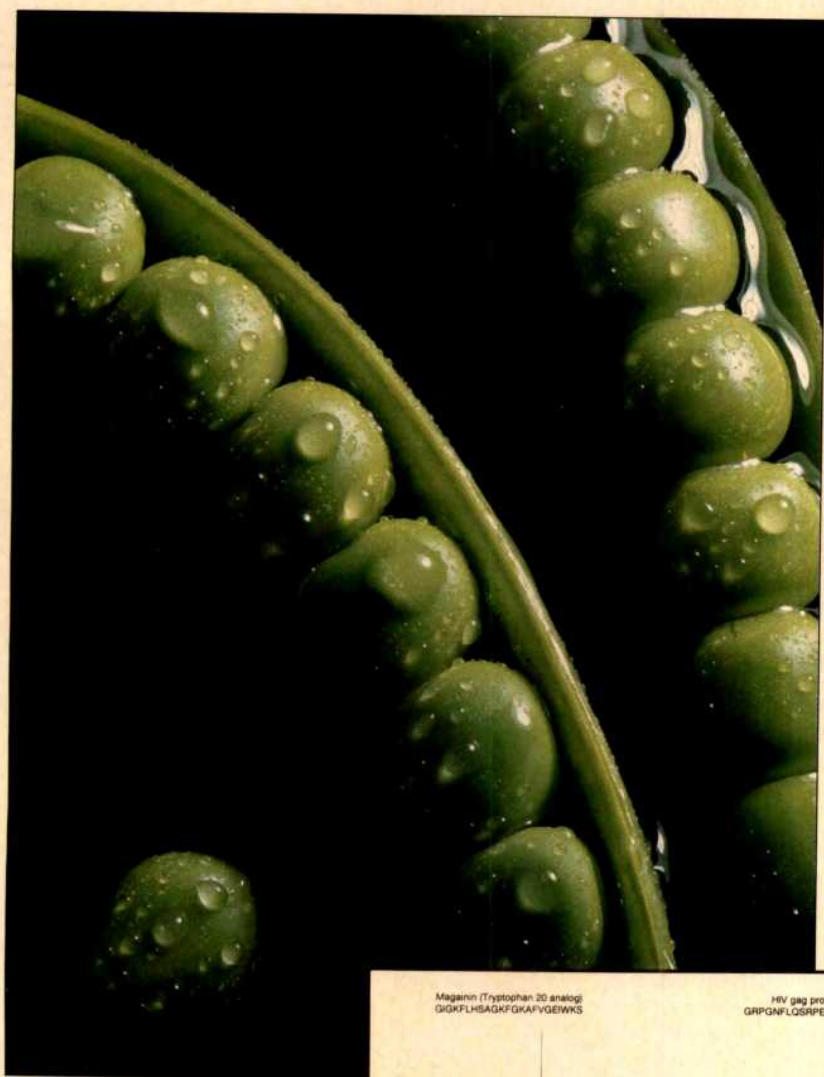
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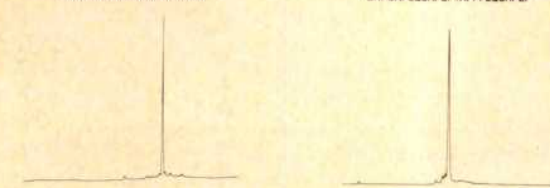
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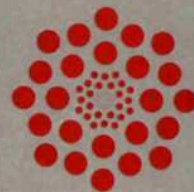


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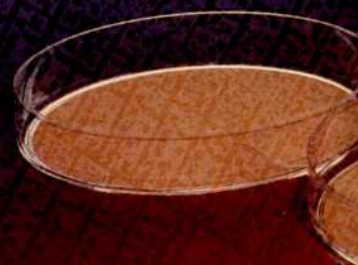
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Efficiencies

- ▶ Generate the most complete libraries
- ▶ Obtain those "non-clonable" inserts

Once again Stratagene breaks new ground by introducing Gigapack™ II lambda phage packaging extract. All mcrA, mcrB, mrr, and hsdR restriction activity has been eliminated from our Gigapack II extracts<sup>1</sup>. The mcrA and mcrB restriction systems of *E. coli* will damage DNA containing 5-methylcytosine. Since 5-methylcytosine occurs naturally in genomic DNA from plant and animal cells, many genomic regions are difficult or impossible to clone. Lambda phage packaging extracts generally contain residual mcrA, mcrB, mrr and hsdR activity. Gigapack II packaging extracts do not contain this activity. The elimination of mcrA and B restriction systems greatly increases the representation and size of lambda and cosmid genomic libraries, especially when using DNA from species having a substantial degree of methylation. Moreover, there are genomic regions in most species that are highly methylated and thus difficult or impossible to clone. Elimination of hsdR restriction activity allows the cloning of inserts bearing Eco K restriction sites, which are not protected by 5-methylcytosine.

Gigapack II packaging extract is also useful for cloning cDNA that has been synthesized using 5-methylcytosine, such as with Stratagene's new unidirectional ZAP™ cDNA synthesis kit. Gigapack II is simple to use and is available in the following kits:

NAME	FEATURES	REACTIONS	CAT#
Gigapack II Gold	2x10 <sup>9</sup> pfu/μg λ DNA	11	200216
Gigapack II Plus	1x10 <sup>9</sup> pfu/μg λ DNA	11	200213
Gigapack II XL	Designed for size selective packaging	11	200219

New restriction minus *E. coli* strains are also available to further improve library representation. The mcrA<sup>-</sup> and mcrB<sup>-</sup> P2 lysogen strain, P2PLK17, permits use of the efficient Spi<sup>-</sup> selection system for low levels of nonrecombinant background clones in genomic library preparation. PLK-F<sup>-</sup> and PLK17 are available for generating efficient unidirectional cDNA expression or subtraction libraries.

Gigapack II is even more effective with methylated DNA using Stratagene's new restriction minus plating hosts CPLK and P2CPLK, which are derived from *E. coli* C.

<sup>1</sup>Kretz, P.L. and Short, J.M. (1989) *Strategies* Vol. 2 No. 2

NAME	CHARACTERISTICS	CAT #
P2PLK17	RecA <sup>+</sup> , mcrA <sup>-</sup> , mcrB <sup>-</sup> , Spi Selection, lac <sup>-</sup> , hsdR <sup>-</sup> M <sup>+</sup>	200290
PLK-F <sup>-</sup>	RecA <sup>-</sup> , mcrA <sup>-</sup> , mcrB <sup>-</sup> , hsdR <sup>-</sup> M <sup>+</sup> , F' (lacZ Δ15, lacI <sup>q</sup> )	200282
PLK17	RecA <sup>+</sup> , mcrA <sup>-</sup> , mcrB <sup>-</sup> , lac <sup>-</sup> , hsdR <sup>-</sup> M <sup>+</sup>	200291
CPLK	RecA <sup>+</sup> , lac <sup>-</sup> ( <i>E. coli</i> C)	200292
P2CPLK	RecA <sup>+</sup> , lac <sup>-</sup> , Spi selection, ( <i>E. coli</i> C)	200293



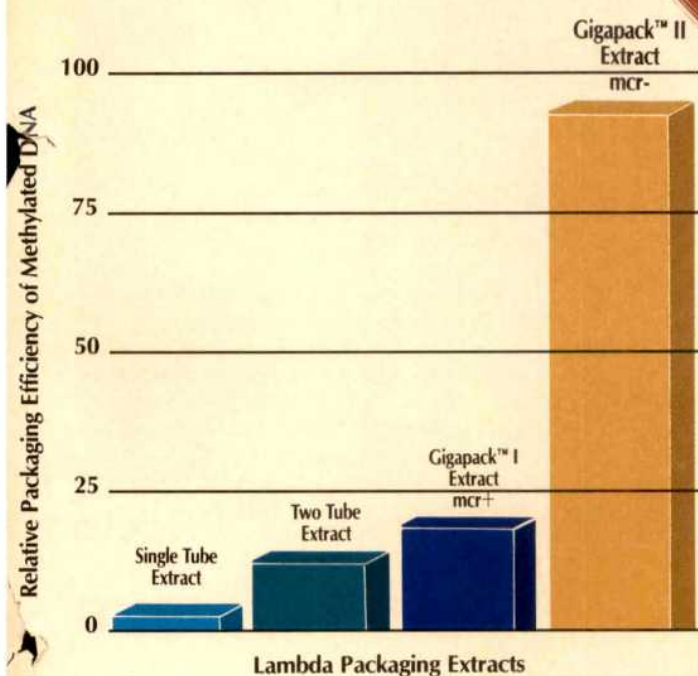


Figure Legend:

Highly methylated eukaryotic DNA was packaged with Gigapack II extract and three other commonly used lambda packaging extracts. The packaged DNA was then plated with the methylated cytosine restriction minus (mcr<sup>-</sup>) *E. coli* strain PLK17. The relative packaging efficiencies are shown.

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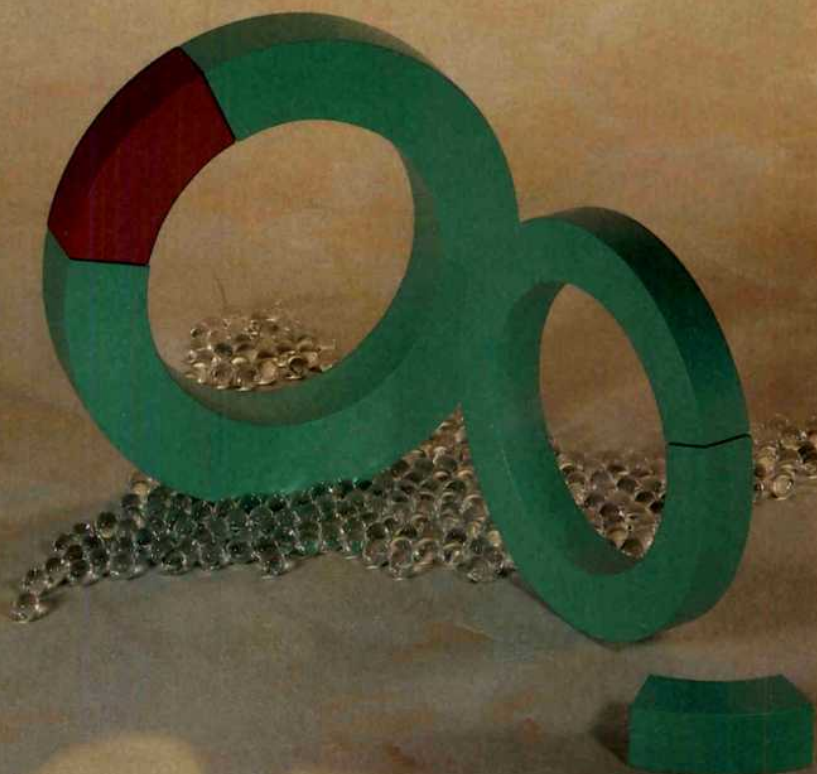
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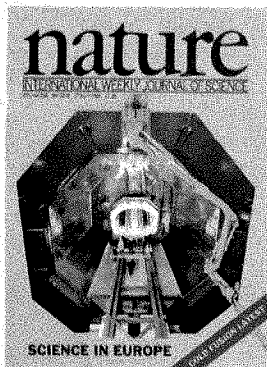
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# nature

27 April 1989

Vol. 338 Issue no. 6218

The cover shows the installation of part of a magnet assembly in the 'L3 experiment' at CERN, one of the European laboratories covered in this week's Science in Europe supplement, on pages 717-736.

## THIS WEEK

### Fusion in print

"When a current is passed through a palladium electrode immersed in an electrolyte of deuterated water and metal salts, a small but significant flux of neutrons is detected. Fusion of free deuterons within the palladium lattice may be the explanation." Read on, on page 737. Discussion of the cold fusion phenomenon continues in News and Views (pages 701, 705 and 710), Scientific Correspondence (page 711) and News.

### Neanderthal behaviour

A rare fossil find from Mount Carmel in Israel suggests that the morphological basis for speech



was fully developed in Neanderthal man 60,000 years ago, pages 758 and 702. At another archaeological site in Israel, radiometric dating sheds new light on the question of whether Neanderthals coexisted with modern man, page 756.

### Multiple sclerosis

A new study of sera and cerebrospinal fluid from thirty patients casts doubt on a previously proposed association between the paramyxovirus SV5 and multiple sclerosis, page 769.

### An ill wind

Monsoon conditions in the Indian Ocean are related to the high biological productivity of the Arabian Sea — and to global carbon dioxide. Page 749.

## AIDS incubation time

An up-to date figure for the mean incubation period for AIDS is provided by new data from analysis of 167 transfusion-related cases in France. The new estimate for the mean incubation time is 5.3 years, with a 90% confidence interval ranging from 4.4 to 8.9 years. Page 768.

## A costly business

In the fruitfly *Drosophila melanogaster*, mating with males greatly reduces lifespan in females whose rates of egg production and egg fertility do not differ. The results presented help to provide a better understanding of the cost of reproduction, page 760.

## An educating epitope?

The first direct evidence that there are conformational differences between major histocompatibility complex molecules found in the thymic cortex and medulla, page 765.

## Chemical bicentenary

Lavoisier's *Traité élémentaire de chimie* set the agenda for much of the work that followed. George Kauffman puts the case for Lavoisier as the founder of modern chemistry. Commentary, page 699.

## Malaria prevention

A human monoclonal antibody prevents knobless malaria-infected erythrocytes from adhering to blood vessels, contradicting the belief that such adhesion requires the knobs that are characteristic of infected cells and suggesting an approach to the prevention of cerebral malaria, page 763.

## Guide to Authors

See Vol. 338, 13 April 1989, page 598.

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NATO should modernize its weapons ■ British newspapers must reform 689-690

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Fossils from the Miocene of Abu Dhabi Henry Gee 704  
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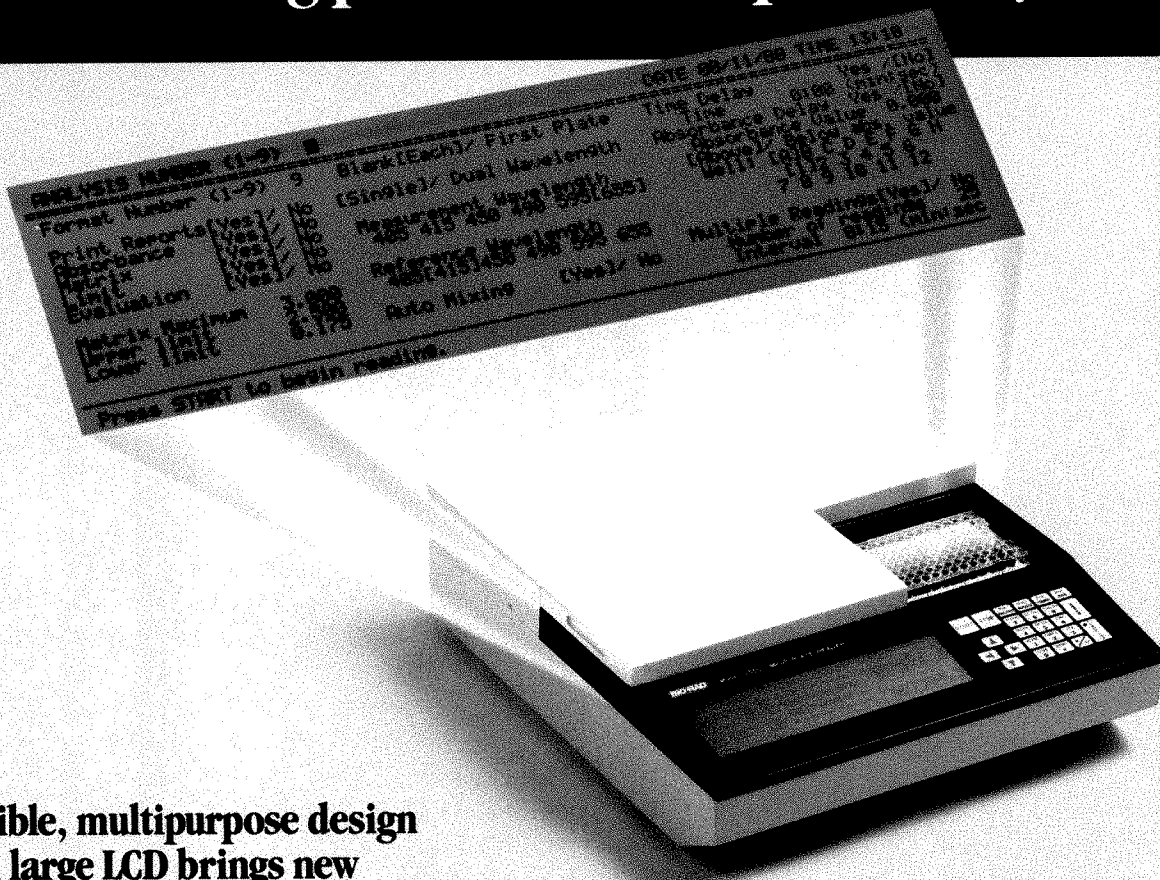
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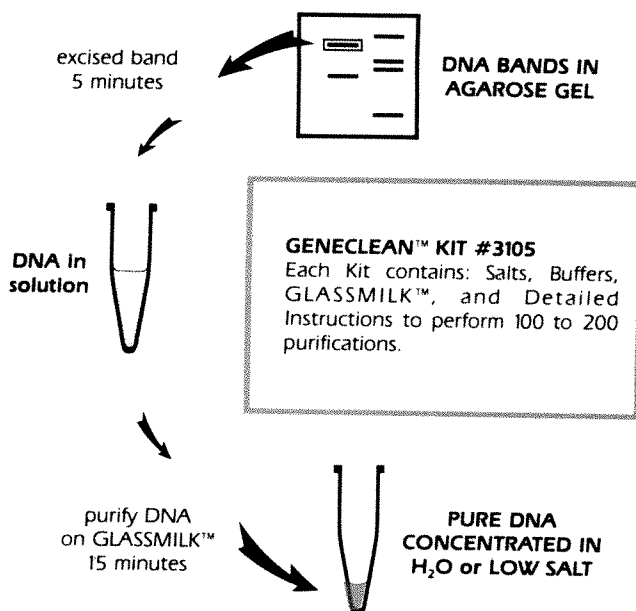
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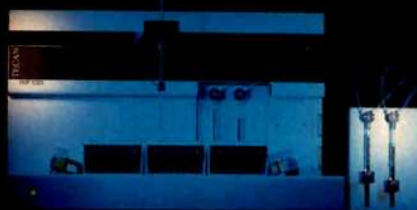
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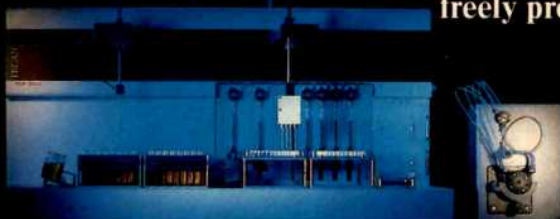
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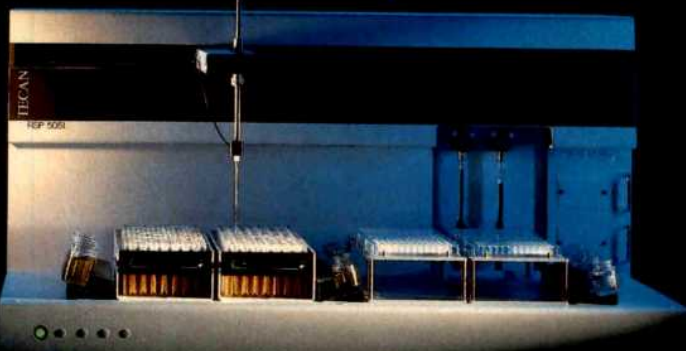
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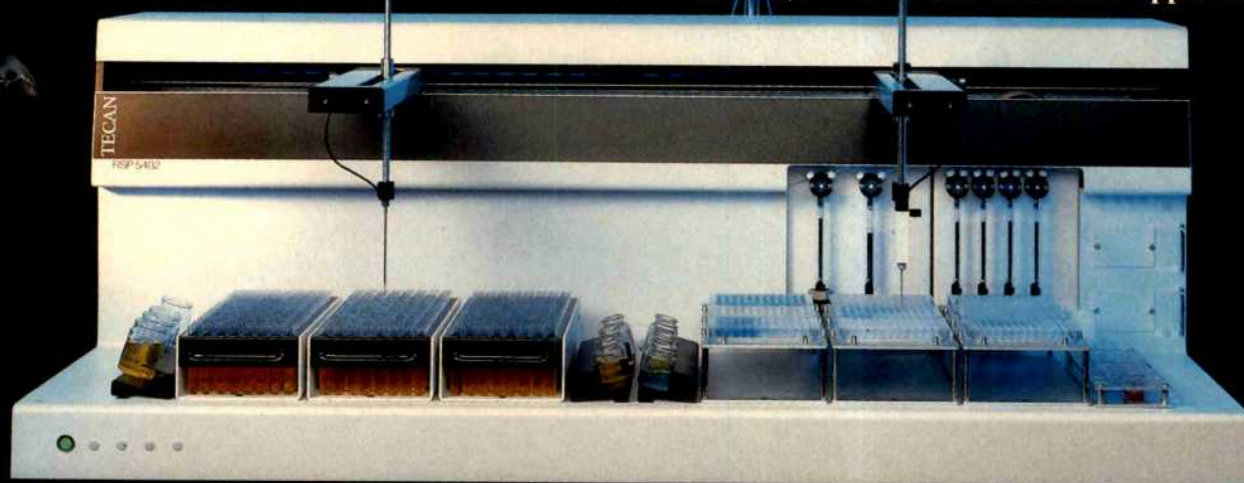
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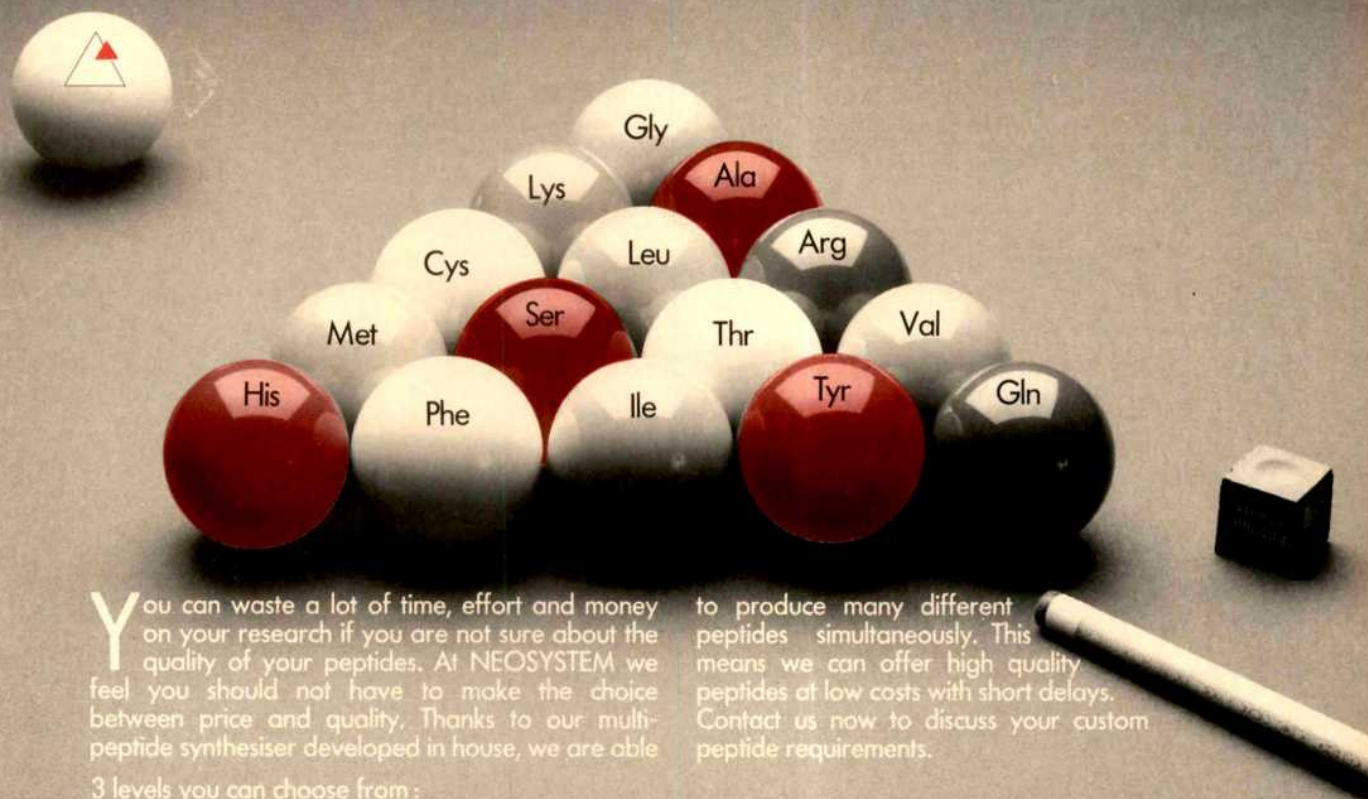
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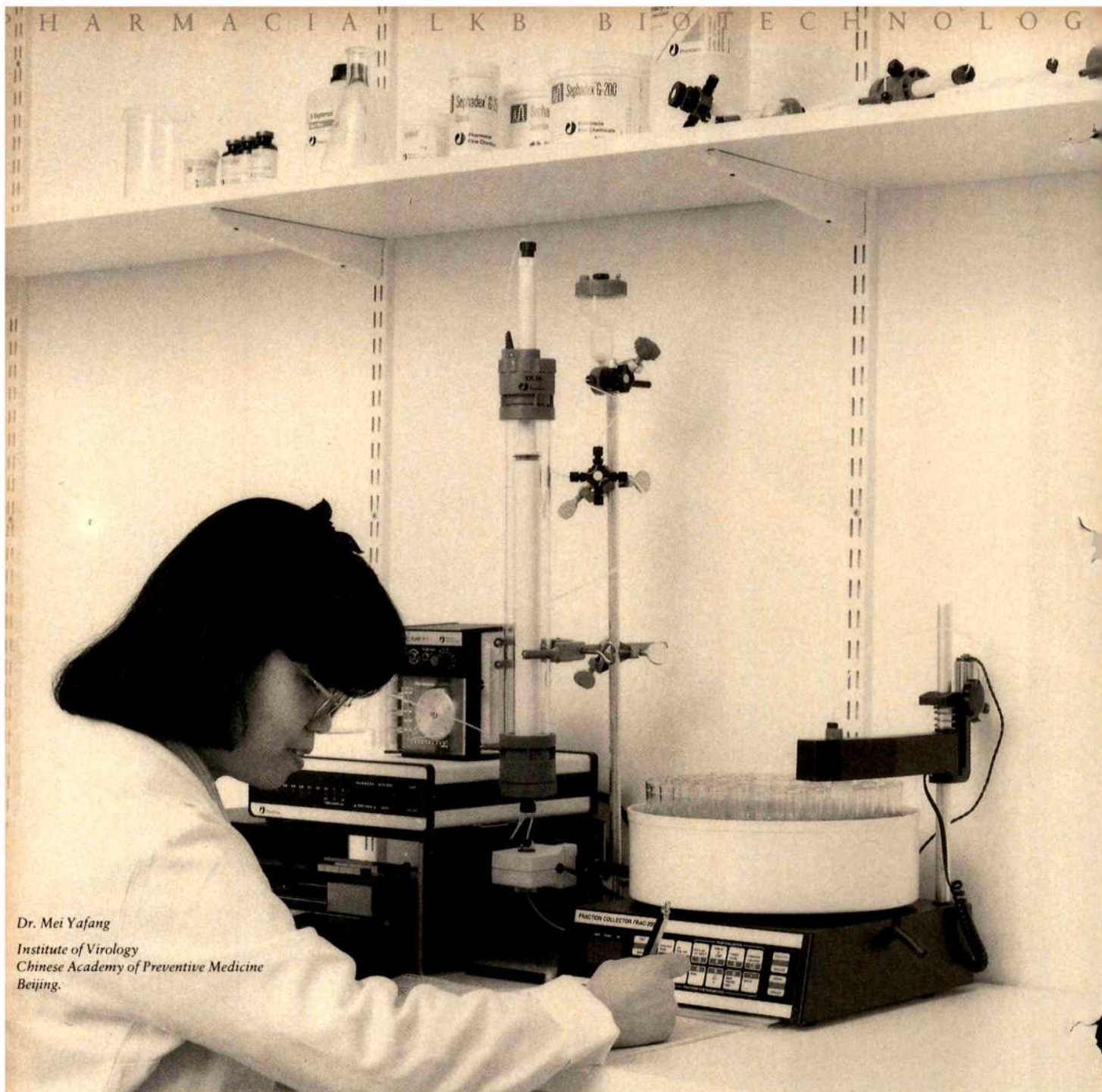
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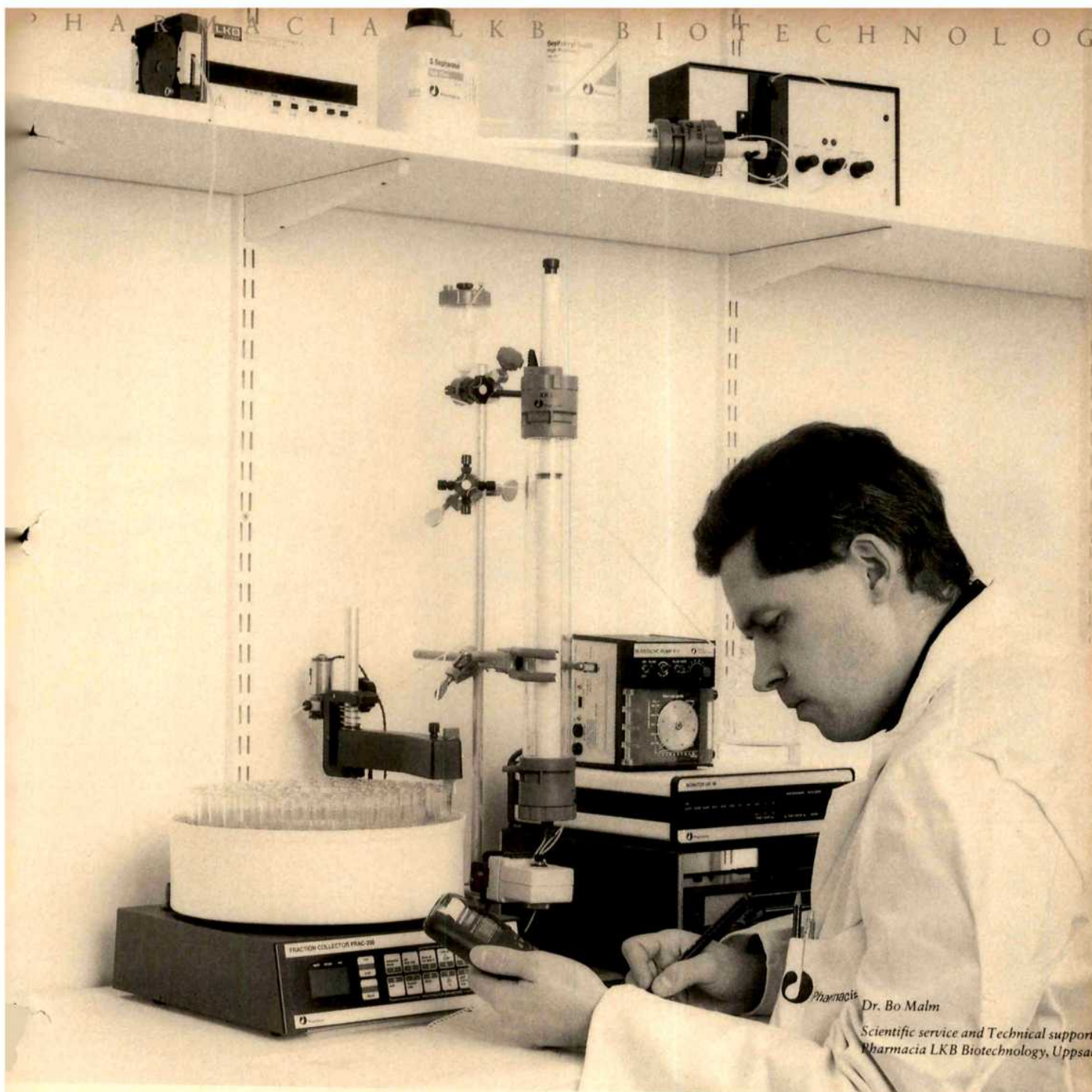
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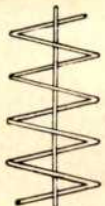
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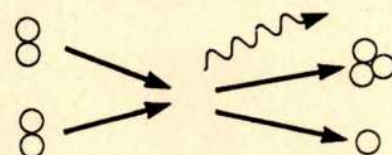
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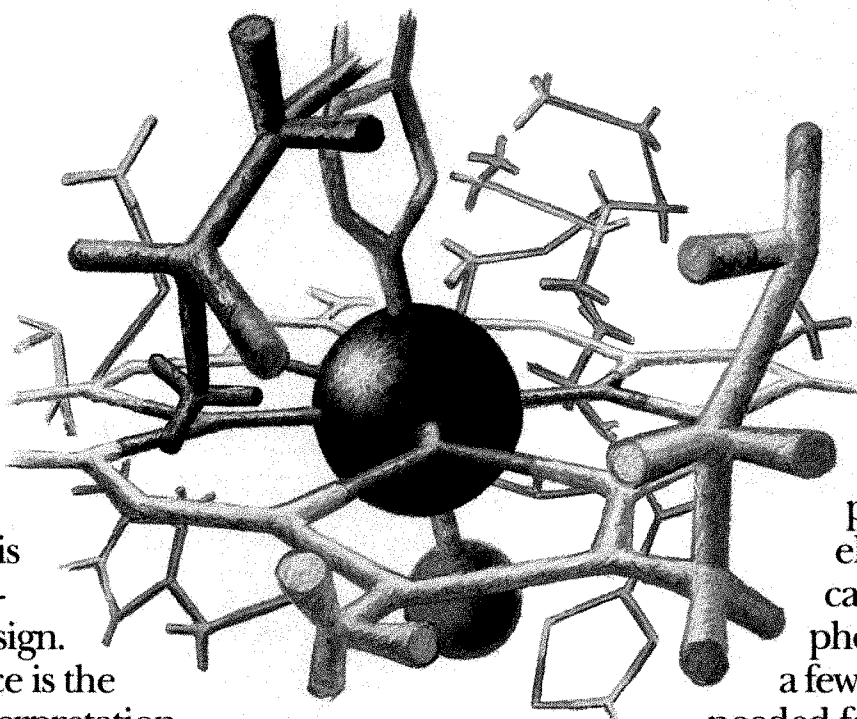
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## How to lose with nothing to lose

NATO is making heavy weather of battlefield weapons in Europe. It would find it easier to modernize its forces if it would negotiate as well, and that way would also keep its friends.

Mr Dick Cheney, the new US Secretary of Defense, will not win many friends in Europe with his statement at the weekend that if the West were to embark on negotiations on short-range nuclear weapons, "there's a very real danger" that the end result would be a "de-nuclearized" Europe. "What would be so wrong with that?" is how many people will respond. Especially if there is also a cast-iron deal on the numbers and character of the conventional forces deployed in Europe by the Warsaw Pact and the North Atlantic Treaty Organization (NATO). In short, Cheney's remark may serve only to strengthen the growing body of opinion in West Germany whose clamour sent Hans-Dietrich Genscher, the Foreign Minister, and Gerhard Stoltenberg, the Defence Minister, scurrying from Bonn to Westminster to put the case for negotiations.

NATO, and the United States and Britain in particular, are in danger of talking themselves into a false position on this issue, which is neither new nor obscure. NATO's European strategy, which goes back to the early 1960s, is explicit, even correct. Supposing (or at least believing) that the Warsaw Pact's forces are the stronger on the ground, Western strategy has relied on battlefield nuclear weapons to beat back an overwhelming conventional attack. But now there are serious negotiations under way at Vienna on the mutual regulation of conventional forces, launched propitiously by the Soviet Union's unilateral decision last December to reduce its armed forces by 500,000 men and women and to withdraw 15,000 tanks from the European region. NATO is well within its rights to say (as it does) that those reductions will not make a stable Europe, and to ask for more to match whatever reductions are offered by the West. But if there is a chance of reaching an acceptable balance on conventional forces, and if short-range nuclear weapons are meant to make up for the West's perceived deficiencies in that area, it must make sense to talk about both at the same time.

So why is NATO so hesitant? One consideration is its wish to replace some of its battlefield weapons with modernized Lance missiles (with a range of 500 kilometres) and, no doubt, to remove many others from Europe altogether. The West German government has been a reluctant partner in that process because many of those who elected it to office would prefer negotiations to modernization, and might elect another government next

spring if they do not get their way. Sympathetic to Chancellor Helmut Kohl's difficulties, some other NATO members have been saying that the decision about Lance could be postponed until after the elections. That explains why the NATO communique last week spoke only of bringing battlefield nuclear weapons "up to date", without saying how or when. West Germany agreed. The significance of the form of words has since been fully explained by NATO governments, Cheney's among them: it amounts to a postponement of a decision about Lance until after the West German elections. Yet West Germans read newspapers as avidly as anybody. If Genscher, one of the minority Free Democrats in the West German Cabinet, has concluded that this form of words, and its intention, has provided the opposition parties with a blunderbuss, that merely shows that he is an experienced politician. No wonder he wants a new understanding.

NATO's fear of negotiations on battlefield weapons is harder to understand. Cheney spoke as if negotiations could have only one end-point: abolition. The logic is elusive. Who believes that the conventional arms negotiations will ban tanks from Central Europe? Why cannot the West appreciate that negotiations on battlefield nuclear weapons could, instead, be an opportunity to convince the Warsaw Pact (and even West German electors) that Lance missiles, being less in danger of conventional capture than, say, nuclear artillery, might be the best embodiment of Western weapons?

Cheney also seemed to argue at the weekend that negotiations are to be avoided because the Soviet Union has always wanted a "de-nuclearized Europe". It is true that Mr Mikhail Gorbachev has sometimes echoed his predecessors in saying that. But what Gorbachev has recently been asking is that there should be negotiations. His argument is cogent (see *Nature* 338, 527; 13 April 1989), and deserves a constructive answer. As time passes, Cheney will find it easier to say "yes" than "no".

The unspoken part of the dialogue is that which affects the nuclear weapons of European states themselves, Britain and France. So far, neither's nuclear planning has been much affected by arms control negotiations. The unratified SALT II treaty, which constrained allowable numbers of Soviet and US strategic missiles, implicitly took account of these peripheral forces, but without direct commitment by their operators: ironically, the



moral force of the treaty is such that the major powers abide by it, but Britain and France can build what weapons they choose. The British Prime Minister, Mrs Margaret Thatcher, has said she will consider negotiating about British weapons when the major powers have a strategic agreement, which will not be tomorrow. Her French counterparts have said nothing. Yet each of them will also have to say "yes" at some stage. □

## Oppressed press

Lacking constitutional protection, British newspapers are under pressure to reform — or be regulated by law.

AMERICANS concerned with press freedom are constantly surprised to find the British government banning books and suppressing news. No doubt that is why, last week, Mr Dana Bullen, the former *Washington Star* journalist who heads the World Press Freedom Committee, told an International Press Institute conference (called to discuss growing press censorship in Britain), that much of the new British Official Secrets Act is "unconstitutional".

Such parochialism, in the days of *Spycatcher*, is no longer excusable. Britain does not have a First Amendment any more than it has a Grand Canyon or a President. The US Congress may pass no law restricting freedom of the press. The British Parliament can. One private member's bill, which would have given a statutory right of reply to those who claim to be victims of inaccurate reporting, was rejected last week. Another — protecting privacy — is expected also to fail. But there is to be an independent inquiry into the conduct of British newspapers. The press, said Mr Timothy Renton of the Home Office, announcing the inquiry, has a year or two "to clean up their act". If not, Parliament will do what Congress cannot.

In last week's debate, much rage was vented against the newspapers, particularly the tabloids. But Renton, among other top Tories, expressed a deep reluctance to introduce restrictions of a kind not seen since the time of Cromwell. The Thatcher government, nonetheless, shows no inhibition at all in trying to censor the much-more-regulated medium of broadcasting. As things are, shocking as it is to many outside Britain, legally elected representatives of the legal Irish nationalist organization, Sinn Fein, including one elected to the Westminster Parliament, are banned from British radio and television. (At least, their voices are: pictures may be shown, with others speaking their words.) But the anger at the supposed damage done by the media could be extended to curbs on the press.

Those who deplore this prospect should keep in mind three factors that do not exist in the United States. One is active terrorism, ready to strike at any time. The Prime Minister was nearly killed by the Provisional IRA at Brighton in 1984 (and five of her colleagues perished). Earlier, one of her closest friends had been killed by a bomb within the precincts of Parliament itself. The second

factor is that there is a large number (eleven) of national newspapers, engaged in a bitter circulation war with each other; many will do anything to gain an advantage over their nearest rival. The third is the royal family. These unfortunate people, unlike pop stars or television personalities or politicians, are ordinary in every way except birth. Yet the smallest detail of their private lives can sell millions of papers. Privacy laws, such as those in the United States, protect people not in the public eye, on the assumption that celebrity is somehow voluntary. It will be hard in Britain to draw up comparable laws because of this royal anomaly.

Hope that legislation may be avoided lies in the intellect and personality of the new chairman of the Press Council, Mr Louis Blom-Cooper, QC. An ardent admirer of the First Amendment and a civil libertarian lawyer of international repute, Blom-Cooper has, for the past few months, led the British newspaper and magazine industry's own voluntary self-appointed regulatory body. He has sworn to make the council more responsive to public alarm. Last week he made an excellent start, by deciding that the council will conduct its own immediate inquiry into the photographic coverage by the tabloid newspapers of the Hillsborough disaster, when close on 100 people were crushed to death in a football stadium. The front-page full-colour close-ups of the faces of dying teenagers distressed many people.

The serious national newspapers — the broadsheets — consider themselves unjustly blamed for the sins of the sleazy tabloids, all lumped together as the unloved 'press'. But the so-called 'qualities' are not blameless. They seem arrogant. They do not carry correction columns of routine errors. They do not have ombudsmen to whom the public may take its grievances. And they do not editorially scold the tabloids when these — as Mr Rupert Murdoch's do — refuse to publicize the rebukes the Press Council gives them.

The British government is not blameless either. It has done nothing to prevent the concentration of newspaper ownership, which only intensifies the tabloids' circulation wars. Murdoch, who already owns *The Times*, the *Sunday Times*, the *Sun* and the *News of the World*, was allowed also to buy *Today* without the sale being referred to the Monopolies and Mergers Commission. And the Prime Minister has bestowed a knighthood on the editor of the *Sun*, apparently forgiving him for its pornographic daily page three because of his paper's staunch Toryism.

The best outcome of the rocky year ahead would be for the Press Council and the independent inquiry to persuade the national newspapers that they must make themselves visibly more responsive to public complaints. That might stave off worse. Bullen last week supposed the virtues of the US system to be so plain that it must have been copied elsewhere. But it has not been. The British Parliament should not make laws telling British newspapers what and what not to print, but is in a mood to do so, and there is no constitution to prevent it. British newspapers, beware. □

# Hopes for nuclear fusion continue to turn cool

■ Press conferences continue

■ Verification mostly halting

## Washington

If the withdrawal by Stanley Pons and Martin Fleischmann of the cold fusion paper submitted to *Nature* bolstered rising scepticism, enough happened this week to keep hopes alive. Claims of successful fusion arrived from California, India and Brazil, and Pons himself hinted at new experimental evidence. But at the press conferences at which the announcements were made, hard data have been lacking and conclusions unclear; still no verdict can be reached.

One positive note is the publication in this issue of the more modest claim by Steven Jones and his colleagues, at Brigham Young University, who give evidence for a small but significant increase in neutron flux when current is passed through a palladium cathode in a suitable electrolytic cell. Jones and his group believe that fusion is occurring, but at a level much below what Pons and Fleischmann need to explain their energy production rate.

That claim is now at the centre of the debate. Neither in the published paper nor in the version submitted to *Nature* is there enough detail for readers to make their own estimations of the workings of the electrolytic cells. That and some other relevant issues are dealt with elsewhere (see pages 701, 705, 710 and 711).

At a press conference in Salt Lake City on 17 April, Stanley Pons announced a qualitatively new piece of supporting evidence: mass spectroscopy of the gases evolving from a working fusion cell revealed the presence of  $^4\text{He}$  in quantities consistent with the reported energy production, if all deuterium-deuterium fusions produce  $^4\text{He}$  rather than tritium and a proton or  $^4\text{He}$  and a neutron.

Cheves Walling, a colleague of Pons, says that gas from the cell was sampled and analysed, and a production rate of  $10^{12}$  atoms per second was deduced after the total gas flow rate had been figured in. This rate of helium production is much greater than in the 1926 'cold fusion' experiments (page 692), in which about  $10^{10}$  atoms were detected after several hours.

Walling and a colleague, John Simons, have submitted to the *Journal of Physical Chemistry* a theoretical paper suggesting how the high density of electrons in the palladium lattice could allow the high-energy photon expected, with a  $^4\text{He}$  nucleus, to be converted into lattice vibrations and thus heat. Although Walling

describes the theory as 'qualitative', he says that enhancements by many orders of magnitude of this normally rare fusion mode could be achieved.

At the 17 April press conference, Pons addressed an issue which has caused disquiet since it was brought up by Harold Furth, director of the Princeton University Plasma Physics Laboratory, at the recent meeting of the American Chemical society in Dallas, Texas (*Nature* 338, 605; 1989) — the lack of a direct comparison between an electrolytic cell containing heavy water and one containing ordinary water. Pons tantalized his audience by indicating that preliminary results from just such a comparison suggested an "unexpected" production of heat in the ordinary cell. There are no numbers.

A similar comparison between 'light' and 'heavy' electrolytic cells was described at Stanford University on 18 April by Robert Huggins, of the Materials Science Department. He said that when two cells, identical except that one used  $\text{H}_2\text{O}$  and the other  $\text{D}_2\text{O}$ , were run side by side with the same voltage applied, the heavy-water cell consistently ran the hotter by as much as 15 per cent. Huggins says that differences in the specific heat of heavy and light water, or in the diffusion rates of hydrogen and deuterium into the palladium electrode, are not large enough to explain the results.

Other groups are attempting similar comparisons, but none has yet reported success; Huggins says that there are "a couple of features" in his set-up that others may have missed, but these will not be revealed until a special session of the meeting of the Materials Research Society, scheduled for the evening of 26 April in San Diego.

In India, two groups claim to have achieved cold fusion in electrolytic cells similar to those used at the University of Utah. A third group, at the Bhabha Atomic Research Centre (BARC) in Bombay, is setting up a large-scale electrolysis reactor expected to produce results towards the end of May.

C.K. Mathews and colleagues at the Indira Gandhi Atomic Research Centre (IGARC) at Kalpakkam near Madras have reported neutron production from electrolysis of heavy water using a platinum anode and a titanium cathode. The team used an electrolyte of nickel and palladium chlorides at 0.2 per cent concentration rather than the lithium salt used by Pons and Fleischmann. "We observed a

30 per cent increase in neutron flux over the background level, suggesting that fusion was taking place", said IGARC director Dr C.V. Sundaram. No measurements were made of energy output. Sundaram said the results were not always reproducible because the experimental parameters had not been optimized.

In an almost identical set-up at the Tata Institute of Fundamental Research in Bombay, researchers claimed to have observed energy amplification, but they did not look for neutrons. Professor K.S.V. Santhanam and his colleagues in the chemical physics group said that passage of 0.25 watts of electrical power through the cell produced one watt of thermal output at the titanium cathode, whose temperature rose to  $80^\circ\text{C}$  in a sustained reaction. Heavy water mixed with sodium chloride was used as the electrolyte and platinum as anode in this experiment.

BARC's director, Dr P.K. Iyengar, said his group is setting up a much larger version of the Utah experiment with sophisticated instrumentation for measuring heat as well as fusion products. Results are expected in a month. But Iyengar, a well-known reactor physicist, is sceptical.

India is one of the few countries producing heavy water in commercial quantities and may therefore have an advantage if cold fusion is real. BARC is also interested in cold fusion as an inexpensive neutron source, which could be used, instead of fast-breeder reactors, to transmute India's 360,000-tonne reserve of thorium into uranium-233 fuel. But BARC's spirit has been dampened by reports that, whatever happens inside palladium, it does not produce a large neutron flux.

In Brazil, two groups have announced, by the now-traditional press conference, that they have obtained fusion reactions while trying to replicate the Utah experiments. The first team to report, from the Physics Institute of the University of São Paulo, said on 19 April that it had detected neutrons at twice the background level. Spero Penha Morato says that only a deuterium-deuterium reaction could account for this excess of neutrons, but the team was not equipped to measure heat production.

The second announcement, on 29 April, from the Institute of Space Research (INPE) in São Jose dos Campos in São Paulo state, says that a group headed by Gerson Otto Ludwig and known for work on fusion by magnetic confinement has reported two separate neutron bursts during a 100-hour experimental run. The first burst, after ten hours, had an intensity of ten times background; the second, after 35 hours, four times. At another press conference on 21 April, the same group announced it had detected  $^3\text{He}$ , thus proving that fusion reactions had occurred. □

# 1920s discovery, retraction

## Munich

WHILE researchers around the world spend sleepless nights in the laboratory trying to reproduce evidence of cold fusion, reports from the United States of a 1926 'mystery paper' on cold fusion in the German-language literature have sent more reflective physicists scurrying to their libraries.

Paul Allison and Klaus Lockner, of Los Alamos National Laboratory, began the chase by sending out an informal translation of a paper from the respected German researchers Fritz Paneth and Kurt Peters, in the 1920s at the Chemical Institute of the University of Berlin, reporting the creation of helium from hydrogen using a palladium catalyst. But the results were retracted eight months later, after new sources of error were identified.

The episode exhibits striking parallels to the current furore over cold fusion. The theory of the day could not explain the results of Paneth and Peters, but there was good reason at least to attempt the transmutation of hydrogen into helium. And the news caused great excitement, as indicated by the response in *Nature* (see excerpts reprinted on page 706).

Little was understood of thermonuclear fusion in 1926, although Paneth and Peters do mention in their introduction to their paper the hypothesis that helium is produced from hydrogen in stars. But neutrons were discovered only in 1932, and physicist Hans Bethe demonstrated in 1933 that fusion was the likely source of stellar energy.

Paneth and Peters published their results first in the journal *Berichte der Deutschen Chemischen Gesellschaft* (59, 2039; 1926). The results were then reprinted in *Die Naturwissenschaften* (14, 956; 1926). This paper and its 1927 retraction (*Berichte der Deutschen Chemischen Gesellschaft* 60, 808; 1927), reprinted in *Die Naturwissenschaften* (16, 379; 1927) are models of clarity. A letter of retraction was also sent to *Nature* (119, 706; 1927).

The technique for the spectroscopic detection of amounts of helium as small as  $10^{-8}$  cubic centimetres was by far the most difficult part of the experiment, and had taken 'several years' to develop. The first stage, in which helium was 'created' from hydrogen, was more a shot in the dark, although palladium was recognized as a catalyst.

At first, Paneth and Peters passed about 1 litre of hydrogen gas through a red-hot palladium capillary to "create" helium "spontaneously". But when they noticed that spectral lines of helium could be seen even when the capillary was at room temperature, they simplified their apparatus. They exposed hydrogen to a number of palladium preparations — a 'black', a

sponge or palladinized asbestos — for various periods of time. After twelve hours, enough helium was formed to show four or five spectral lines.

Paneth and Peters tried hard to account for possible errors in their method. For example, helium might have been trapped inside the palladium. They convinced themselves that this could not be so by repeatedly exposing their palladinized asbestos catalyst to hydrogen and oxygen. Only in the presence of hydrogen was helium released. Therefore, they concluded, the helium must have derived from hydrogen and not from an experimental artefact.

Unlike Pons and Fleischmann, Paneth and Peters did not observe the release of large amounts of heat from their apparatus. They write that they would have expected only a fraction of a calorie of heat to be produced by the creation of  $10^{-8}$  cubic centimetres of helium. Paneth and Peters conclude that the energy must be released in the form of radiation, but add that they had not detected it.

In April 1927, came the retraction. Paneth *et al.* had tested their results at Cornell University and in Berlin and drew the conclusion that they had "underestimated" two sources of error.

The first clue emerged during experiments designed to check whether helium could have diffused from the atmosphere through the glass walls of the apparatus. While performing numerous control studies, Paneth *et al.* found that glass heated in a hydrogen atmosphere yielded up absorbed helium, in amounts of about  $10^{-9}$  cubic centimetres, whereas glass heated in a vacuum yielded none. Helium detections at this level, they concluded, were to be discounted.

The second blow was the realization that the palladinized asbestos catalyst that had given the best results was, like glass, a considerable source of helium, which it released readily in the presence of hydrogen, but not in that of oxygen. In an almost self-mocking tone, Paneth *et al.* write that they must strike from their results all the trials with a palladinized asbestos catalyst, in which helium was 'created' in amounts up to  $10^{-7}$  cubic centimetres, and upon which they had earlier placed "particular value".

The story also has an addendum which parallels modern activities. In February 1927, John Tandberg of the Electrolux Research Laboratory filed for a Swedish patent on a device which produced "helium and useful energy". This invention was an electrolytic cell, using ordinary water, based on the work of Paneth and Peters but with a "significant increase in efficiency". The patent was never granted.

Steven Dickman

## Complicity alleged against physicist

### Munich

A Max-Planck Society physicist has been implicated in illegal exports to Pakistan of sensitive nuclear technology. Testimony given before a parliamentary committee in Bonn on 20 April revealed that the physicist, a prolific inventor who received a number of patents for himself and his employer, the Max-Planck Institute for Plasma Physics (IPP) in Garching, may be prosecuted for his involvement in the exports. He was dismissed from his post on 17 April when the institute's directors learned the extent of his involvement.

It had been known since December that Pakistan, South Africa and India had received nuclear technology from the West German companies NTG (Neue Technologien GmbH) and PTB (Physikalisch-Technische Beratung) without the necessary export licences having been issued by the West German government.

The physicist was also mentioned in the news as a consultant for NTG. But IPP defended him at the time, saying that his consulting work did not go beyond installing a tritium-removal apparatus using the so-called TROC process (Tritium Removal with Organic Compounds). TROC is used to remove tritium from glove boxes and other work areas. It cannot be used for the production or holding of the large amounts of tritium that can intensify the effect of a nuclear bomb. The physicist had developed TROC and IPP holds the patent on it.

But last week it emerged that the physicist also helped NTG install a "tritium handling system" for holding large amounts of tritium free from contamination. The use of this system for bomb production cannot be ruled out, though by itself it cannot produce tritium. The physicist did not inform IPP of his activities nor did he say that he had formed his own consulting firm.

The technology exported to South Africa and India could have been used only for peaceful purposes, said Hanau prosecutor Albert Farwick, who is handling the case against NTG, which is located in Gelnhausen, in the *Land* of Hesse. Pakistan denies that it broke West German export law.

The decision whether to prosecute the physicist will be reached by the autumn, said Farwick, who said that the physicist was "in no way the head" of the export operation. The case has generated some sympathy for the physicist, who is looked upon by some of his IPP colleagues as a "tragic case".

The physicist, who is estimated to have earned about DM50,000 for his work for NTG, is appealing against his dismissal by IPP. His case is expected to be heard in two weeks.

Steven Dickman



## SCIENCE POLICY

## Press pleads for more basic science

Washington

THE new US presidential science adviser D. Allan Bromley is "walking into a situation unparalleled in opportunity", according to US National Academy of Sciences president Frank Press, himself a presidential science adviser during the Carter presidency.

Bromley, Press said, will have a chance to accomplish real advances in the way the US science enterprise is financed and managed. Press, speaking in advance of his annual address to the members of the academy, said that the Bush administration's concern for maintaining US strengths in science and technology, coupled with the increased political power of the science advisory post, could make Bromley's tenure the most impressive in recent history.

Press's hints that the next few years may be a watershed for US science were borne out in perhaps the most sweeping of his yearly speeches to the members of the science academy. In marked contrast with last year's address (*Nature* 333, 1; 1988) in which he stressed the importance of setting priorities for science within the confines of a budget, Press called for doubling US spending on basic science over the next five years to \$20,000 million.

Press says that could be done by raising the growth rate of the basic science budget from 6 to 14 per cent per year, at a total cost to the United States of \$10,000 million. Such a hefty rise should be possible, says Press, if big expenditures are delayed until after the federal budget deficit is reduced.

To remove the barriers blocking the industrial benefits of such a large investment in science and technology, Press proposes the creation of a cabinet-level National Economic Security Council with the same powers over the high-technology economy that the National Security Council has over defence. His view is that the presidential science adviser would play a key role in directing the activities of the council.

Press also advocated a "national facilities act" to provide the infrastructure necessary for major science developments. The legislation would pay for refurbishing laboratories, instruments and equipment. Press estimates the equivalent of 50 new buildings could be had for \$1,250 million. Press also advocates the creation of 3,000 graduate fellowships in science and engineering, at a cost of \$3,000 million.

But Press's grand proposals may not be taken up as enthusiastically as he would wish by Congress and the Bush administration. President Reagan's stated goal to double the US National Science Foundation budget over five years has not yet been attained. But Press has the patience to wait: if his suggestions are not taken up this year, he plans to pursue them again in 1990.

Carol Ezzell

## Presidential adviser named

Boston & Washington

AFTER more than two months of delay and increasingly vocal protest from the scientific community, US President George Bush last week finally announced his choice of Yale University professor D. Allan



D. Allan Bromley, new science adviser.

Bromley for the post of presidential science adviser.

Bromley's appointment continues a tradition of favouring physicists for the post of adviser to the president and director of the Office of Science and Technology Policy. Bromley, who will be 63 next week, is Henry Ford II Professor of Physics and director of the A.W. Wright Nuclear Structure Laboratory at Yale University. He was born in Canada and has a high reputation in heavy-ion physics where he has been active in the development of accelerators, detectors and computer-based data collection and analysis systems.

Bush's failure to keep his promise to select a science adviser early in his administration had already prompted congressional protest by the time of the Bromley appointment. On 6 April, at a hearing of the Senate Labor and Human Resources Committee called by Senator Edward Kennedy (Democrat, Massachusetts), a series of witnesses, including Frank Press, president of the National Academy of Sciences, and two former science advisers, Donald Hornig and Guy Stever, expressed their disappointment that a science adviser had still not been appointed and that few of the 500 key federal administrative posts in science had been filled.

But Bromley says that Bush's campaign promise to upgrade the status of the science adviser is now to be fulfilled. Following "a discussion with the president" on 21 April, Bromley confirmed that "the new post will be the assistant to the president for science and technology. The science adviser will have direct access to the president and will be on the same level as the national security adviser [Brent Scowcroft].... This means that I will participate in meetings of the National Security Council, and I will be present at meetings that had not involved past science advisers." Bromley says he will not try to bring his own priorities to the

job but rather "sharpen options for the president" and "be part of the president's team". "Any science adviser who is perceived as a spokesperson for the scientific community is doomed to failure", he says. "The science adviser must be trusted in all detail by the president, must participate in policy debate, and be present in high-level decisions that have a relevance to science and technological matters." Numerous problems with a strong scientific component face the president, including the need for decisions on the Strategic Defense Initiative, the space station, the Superconducting Super Collider, the project to map the human genome, the space plane, and international action to counter global atmospheric change.

Even more difficult, because the choices are not clear, will be the setting of national policy to restore the United States' technological superiority and industrial competitiveness in the face of the growing strength of Japan and Western Europe. "This issue leads quickly to concern for education and training", says Bromley. "The questions of science and technology today are integrated across a whole spectrum of governmental affairs. It is very different from Eisenhower's day when the position was created. Now, agencies across the board are very sophisticated in their use of science and technology." So many agencies are now involved in science that some experts believe that a science adviser cannot hope to have much impact and that a new science organization is required. In testimony before Senator Kennedy, Donald Hornig, former adviser to President Lyndon Johnson, pointed out that the Office of Science and Technology Policy has never had much influence over the National Institutes of Health, with their \$7,000 million budget, nor over the Department of Defense, where two-thirds of the federal research and development budget (\$42,000 million) is spent.

The present science adviser, William Graham, claims to have spent much time nurturing inter-agency communication. But Graham's soft-spoken style gave him a reputation for near-invisibility. Colleagues of Bromley describe a man who is likely to act very differently, speaking of him as "emphatic", with new students initially "in awe of him".

Bromley directed former President Ronald Reagan's science and technology initiatives with India and Brazil and stresses the importance of international science. A key question, he says, is whether the United States should embark on huge projects "alone as a nation, or whether we should involve other countries".

Seth Shulman & Alun Anderson



# Gupta affirms authenticity

## New Delhi

PROFESSOR V.J. Gupta, of the Panjab University at Chandigarh, has angrily denied charges by Dr John Talent in last week's *Nature* (338, 613; 1989) that he invented fictitious fossils and had rewritten the geological history of the Himalayas through dubious publications. Gupta claimed that Talent's outburst was motivated by "a malicious intent to take revenge for personal rivalry and professional jealousy over the past 20 years".

Gupta did not specify Talent's motives for revenge, but implied that his interest in the Himalayas went beyond geology, and that the attack on him had something to do with information he was unable to provide to Talent. Gupta said his fossil collections came from Himalayan sites that were either militarily sensitive or close to international borders and thus not open to foreigners.

According to Gupta, Talent and his associates "wanted some information [about localities] which we cannot provide [for security reasons]". He said that Talent visited India 20 times "always entering from Pakistan", but says that he did not publish any paper on India. "I wonder which agency would finance a scientist for so many travels that result in no scientific output?" he asked.

On the authenticity of his fossils, Gupta says they were dug out by a team, not by him alone. To get them identified, expert opinions were sought from palaeontologists abroad. In some cases, opinions were contradictory, but "the diverse opinions were kept in view while working out the stratigraphic set-up of the area, taking account of the geological succession exposed in the field from where the fossils were collected". He said Talent was biased in his opinion about the ages of stratigraphic units.

Replying to the charge that his details of the localities at which his fossils were collected are distorted, Gupta said he had had to depend on old and inaccurate maps whose use is restricted by the government for security reasons. Gupta said his 25 years of work has documented the existence of Silurian-Devonian succession in the Himalayas and that "the truth about it will be proved by the progeny during the forthcoming years".

Predictably, the Talent-Gupta controversy has evoked a spate of comments from Indian scientists and hurried meetings at the Indian Geological Survey. Gupta's colleagues at the Centre for Advanced Study in Geology, including its director, Dr A.K. Prasad, described Talent's allegations as "a conspiracy to denigrate a top Indian scientist".

But the Society for Scientific Values (SSV) plans to launch its own investiga-

tion of the allegations. Describing them as "serious and undermining of Indian science", the society's president, Dr A.S. Paintal, said it will appoint an expert team to make an impartial evaluation of Gupta's work.

Contrary to Talent's implied remarks,

## CFCs

# Japan supports ban by the year 2000

## Tokyo

JAPAN will support a total ban on the use and production of chlorofluorocarbons (CFCs) by the year 2000, Japan's Environment Agency announced last week.

Japan's decision, to be formally announced next month in Helsinki at a meeting of signatories of the Montreal Protocol, follows similar pledges to end the use of CFCs made last month by the United States, the European Community and Canada (see *Nature* 338, 101; 1989). Japan's support will be important for the success of a total ban as it is one of the largest producers of CFCs, accounting for about 10 per cent of world production.

The Environment Agency has been keen to support revision of the Montreal Protocol and the announcement represents a victory over the powerful Ministry of International Trade and Industry (MITI). MITI is concerned that Japan's semiconductor industry may be seriously affected by a ban. Japan is the world's largest consumer of freon 113, the CFC used to clean semiconductors, and produces approximately 130,000 tonnes annually. Alternatives to

India does have a mechanism to check fraudulent research, said Paintal, who is also chief of the Indian Council of Medical Research. With a membership of over 120 reputable scientists, SSV was set up two years ago with the aim of improving the quality of science in India. So far, it has been concerned largely with allegations of misconduct in biomedical research.

K.S. Jayaraman

freon 113 are still in the early stages of development.

The machinations that led to the Environment Agency victory are unknown, but the words of an eminent foreign scientist and Japan's emperor may have helped.

Earlier this month, Frank Sherwood Rowland of the University of California, who was in Tokyo to receive the Japan Prize for his pioneering research on the link between CFCs and ozone depletion, repeatedly called for a worldwide ban. His appeals received wide media coverage.

At the presentation ceremony for the prize, the Emperor in a congratulatory speech noted that when CFCs were first synthesized for practical application, no one suspected that they would have such "dire consequences" he said that this should be a reminder that "science and technology need to be constantly monitored and appraised from newer and wider perspectives".

It is unusual for a Japanese emperor to make a public statement on such a politically sensitive subject. Following such a speech it would have been odd if Japan had not supported a ban.

David Swinbanks

## ANIMAL RIGHTS

# Anti-terrorism bill introduced in Senate

## Tucson

CONGRESSIONAL legislation that would make it a federal crime to steal or harm research animals or to break into and damage research facilities was introduced on 7 April in the US Senate.

Alabama Democrat Howell T. Heflin proposed the Animal Research Facility Protection Act of 1989 so that federal law enforcement agencies can become involved against what he said could be an international conspiracy.

During the next month, the Senate Committee on Agriculture, Nutrition and Forestry will review the bill, which will be an amendment to the Animal Welfare Act. Passage into law could take more than a year. The legislation also makes unauthorized entry into facilities and possession, control or duplication of records, data, material, equipment or animals a possible federal offence. Each violation could lead to a one-year prison sentence and a \$5,000 fine. The district court or magistrate could force the guilty party to

pay for damages and for reasonable cost of repeating ruined experiments. The research institution could also sue for damages.

The legislation also calls for the Secretary of Agriculture and the attorney general to assess the extent and effects of "domestic and international terrorism" on animal research, production and processing facilities. That survey would include places where animals are on exhibit or used in food production or as pets. A report would be due within a year.

Heflin said he had been working on the bill for several months at the insistence of the University of Alabama in Birmingham, but that a recent incident at the University of Arizona in Tucson (see *Nature* 338, 534; 1989) "acted as a catalyst to bring about a more rapid introduction than we had planned". In that incident, a group calling itself the Animal Liberation Front set fires in two university buildings, vandalized laboratories in two other buildings and took about 1,200 small animals.

Elizabeth Pennisi

## SCIENTIFIC MISCONDUCT

# New office gets going

## Washington

THE National Institutes of Health (NIH) Office of Scientific Integrity is changing from an administrative requirement into a reality. It has been given space on the crowded NIH campus, an acting director has been appointed and staff are being



assembled. But all this is happening despite threats from Congress that the office should be moved out of NIH to another agency that can be more objective about misconduct in biomedical research.

NIH and other federal agencies have been labouring hard over the past several months to find better ways of coping with scientific misconduct. Although few in the research community believe that the problem is widespread or growing, congressional and public interest has been fostered by several recent well-reported cases of alleged misconduct.

Earlier this year, the Public Health Service decided to create two new offices to deal with misconduct (see *Nature* 338, 588; 1989). The Office of Scientific Integrity in the office of the director of NIH will oversee — and conduct when necessary — misconduct investigations, recommend punishments and promote high standards of research conduct. The Office of Scientific Integrity Review will be in the office of the Assistant Secretary for Health of the Department of Health and Human Services, and will oversee the NIH office.

Brian Kimes, associate director for extramural programmes in the division of cancer biology and diagnosis at the National Cancer Institute, will become full-time acting director of the NIH office on 1 June, and will run the office on a part-time basis until then. Initially there will be a staff of eight, including two secretaries. Kimes expects that it will take about a year to clear the backlog of some 40 misconduct cases that are pending.

Although some cases will always take time to resolve, Kimes says a priority will be to speed up processing of the more routine cases. He also believes that as institutions receiving grants become more

adept at dealing with issues of misconduct — partly through experience and partly from guidance provided by NIH — fewer cases will require attention from NIH.

Kimes has had previous experience in misconduct, having participated in the investigation of Mark Spector, a graduate student at Cornell University. He says cutting-edge research that is somehow adulterated is easy to spot because many laboratories will quickly attempt to replicate it. Far harder to detect and punish is research that lies outside the main stream, published in journals that do not provide careful refereeing of submitted work.

Despite action by NIH, there still exists the possibility that legislation will be enacted to move the office out of NIH and into the office of the assistant secretary for health. Leslie Russell, a staff member of the powerful House of Representatives Energy and Commerce Committee, told an audience earlier this month, at a colloquium on science and technology of the American Association for the Advancement of Science, that such legislation will be introduced soon. Russell said NIH may face a conflict of interest in looking into misconduct research.

New regulations that spell out the responsibilities of federally funded institutions for investigating misconduct will be published in the near future. They are at present awaiting approval at the White House Office of Management and Budget.

Joseph Palca

## EUROPEAN COLLABORATION

# 25 years of ESA

## Paris

THE European Space Agency (ESA), whose headquarters are in Paris, last week added another birthday celebration to those of the Eiffel Tower and the French Revolution. It is now 25 years since European countries officially pooled resources to form the European Space Research Organisation (ESRO) and the European Launcher Development Organisation (ELDO). These merged in 1974 to become ESA (see page 718).

Helmut Kohl, the West German Chancellor, who was in Paris for a Franco-German summit, applauded ESA's success and invited the agency to promote its role in Earth observation, both to further efforts in pollution control and also to monitor progress in disarmament — a role its constitution probably excludes. French President François Mitterrand saw ESA as also a triumph for France, the biggest partner and principal driving force behind the commercial launch sector, Arianespace, as well as the transportation elements (Hermes and Ariane 5) of Europe's Columbus contribution to the US Freedom space station.

Nobel laureate Carlo Rubbia meanwhile reminded the distinguished gathering of scientists and diplomats from ESA's 13 member states that CERN (the European Laboratory for Particle Physics), ESA's 'elder brother' in Europe's family of large organizations, was established by some of the same scientists as was ESRO.

Peter Coles

## CHARITIES

# UK cancer fund finds new ways of making money

## London

THE Imperial Cancer Research Fund (ICRF), one of Britain's largest medical research charities, has substantially increased direct expenditure on research in the 1980s, by increasing its income and eating into its investment reserves. Next year, those reserves are expected to reach £14 million, the minimum desirable, and Sir Walter Bodmer, ICRF's scientific director, now says any new plans for expansion will have to wait for three to four years until income exceeds expenditure. In the annual report, published last week, the treasurer reports a 27 per cent increase in direct expenditure on research last year, which left income almost £7 million less than expenditure, a deficit met from reserves.

Increased income has come from a new initiative — charity shops. Since 1983, ICRF has set up 200 shops, which now bring in £4 million a year. By the late 1990s, the fund hopes to have 600 shops bringing in £16 million and to double income received from legacies to £72 million.

Major new projects under way include new laboratories at the Institute of Molecular Medicine in Oxford, for research into solid tumours; a new laboratory at the University of Cambridge for research into a vaccine for cervical cancer, by the ICRF's tumour virus group; and a new unit at the Institute of Psychiatry at Maudsley Hospital, London, to investigate smoking behaviour as well as examining the effects of passive smoking on children.

Expansion of the ICRF's main laboratories at Lincoln's Inn Fields, in central London, is also under way. A new building for administration and computing facilities will free space for 50 laboratory bays. Conversion into laboratories is due to begin in 1990 and will cost £3.3 million. Uncertainty about the funds that will be available could mean that completion will be slower than planned, a maximum of four years instead of three. But Bodmer says this development has top priority in the, and stresses that no existing commitments will be cut to meet costs of this expansion.

Christine McGourty



## Democracy finally wins

Moscow

ON 20 April, the Soviet Academy of Sciences finally elected its quota of deputies to the country's new parliament, bringing to an end the four-month election campaign to the Congress of People's Deputies that will form the Soviet parliament.

Members of the Soviet Academy had wanted to be represented by socially orientated legislators, people with radical reform programmes in their pockets, who would firmly and boldly struggle against the administrative and command system, rather than talking about *perestroika*.

The election procedure in this public organization has evoked much interest both in the Soviet Union and abroad. In March, the academy's election conference annulled the decision of the extended plenum of the academy's leadership, held last January, to nominate 23 candidates for 20 vacancies and to ignore a long list of names advanced by numerous academic institutes. Then, only eight candidates won the necessary number of votes (at least one more than the number entitled to vote), while the other 12 seats remained vacant. This triggered the legal requirement for the follow-up elections.

The academy's leadership learned the lesson. They took into account proposals advanced by academic institutes, invited the press and the pressure group called "For Democratic Elections in the Soviet Academy". Voting by a show of hands, they nominated 25 candidates for 12 seats. (On the last day before the vote, the well-known Soviet economist, Academician

Stanislav Shatalin, withdrew from the elections for health reasons.)

The election conference was attended by 1,101 voters, including Soviet academicians, non-voting members of the academy and 433 representatives of academic institutes. The voting was preceded by heated debates: all candidates presented their political, social and legal programmes and answered scores of tough questions about the most pressing issues of *perestroika*.

The support of grass-roots organizations proved decisive. Those elected were, among academicians, Andrei Sakharov, Roald Sagdeev, Vitaly Ginzburg and Georgy Arbatov and, among non-voting members of the academy, Sergei Averintsev, Pavel Bunich and Nikolai Petrakov. Other successful candidates were doctors of economics Nikolai Shmelev and Gennadi Lisichkin, both well-known authors of strongly worded articles; Dr Alexander Yakovlev, a lawyer; Yuri Kariakin, head research worker of the Institute of the International Labour Movement; and Dr Viacheslav Ivanov, a philologist.

On balance, the Soviet Academy of Sciences has passed its examination in democracy with flying colours. Undoubtedly, its deputies to the Soviet Parliament as well as scientists elected from other public organizations, territorial and national-territorial districts, will represent a powerful group working to speed up the radical restructuring of domestic and foreign policies.

Yuri Kanin

### NUCLEAR REACTORS

## Superphénix go-ahead

Paris

Superphénix, the French fast-breeder reactor, has been given the go-ahead to begin generating electricity again after a two-year interruption while a structural fault was investigated. Earlier this year, the reactor was restarted without its leaky fuel-rod transit chamber, despite protests from environmental groups in neighbouring Switzerland who want the plant to be closed down.

Following trials without producing electricity, the French nuclear safety authorities are satisfied that the generator can resume full-power output (1,200 MW) within the next two months. But environmental groups are outraged that no independent inquiry has been carried out and say that to restart the generator without the transit chamber will leave the plant with no safe site to allow fuel rods to cool in the event of an accident. A new transit system for fuel rods is being installed and will be ready for the autumn.

Peter Coles

### AIDS

## Controversial visa ban

Washington

The US policy of denying entry visas to foreigners infected with the human immunodeficiency virus, the virus which causes AIDS, may become a contentious issue before next year's international AIDS conference, scheduled to take place in San Francisco, California. Several researchers are expected to introduce a resolution at this year's conference in Montreal, Canada to move the 1990 conference to a country that does not screen travellers for HIV infection.

The United States passed a law two years ago prohibiting immigrants and aliens infected with HIV from routinely entering the country. Several US congressmen have said that the intent of the law was to turn away HIV-infected immigrants seeking permanent residence, but the law recently was invoked to detain a Dutch city official with AIDS who was travelling to the United States for a health conference.

Carol Ezzell

## Test flight of IRBM abandoned after postponements

New Delhi

INDIA'S eagerly awaited test flight of its first intermediate-range ballistic missile (IRBM) did not take place on 20 April as scheduled, disappointing dignitaries including the defence minister and the three service chiefs who had come to witness the launch from Chandipur in coastal Orissa state. The launch time was postponed three times and late in the afternoon the Defence Research Development Organisation (DRDO), which developed the missile, announced that the test had been put off indefinitely. No reasons were given, although unofficial reports said the mission was aborted because of technical problems.

The two-stage, 100-tonne missile named Agni is said to have a range of 2,500 km and to be able to deliver a warhead weighing more than a tonne. Except for the inertial navigation system, for which France provided help, the missile is claimed to be fully indigenous. The first stage is solid-fuelled for quick acceleration and the second is propelled by a storable liquid of very high specific impulse.

Although not officially stated, the main aim of the launch was to evaluate the performance of the heat shield — an important piece of hardware in a weapon delivery system — during its re-entry through the atmosphere.

The missile, in fact, had been named after this important mission objective (*agni* means fire in Sanskrit). Although DRDO had developed the heat shield some time ago, its testing had to wait until Agni was ready.

After Trishul and Prithvi, Agni is the third missile to roll out of the DRDO laboratories in Hyderabad. Trishul is a short-range (12-km) surface-to-surface missile.

The postponement of the IRBM launch after much publicity is a setback to DRDO which had hoped to score over Pakistan's recent success with its HATF-2 missile, which brings Bombay and Delhi within range. Prithvi, DRDO's long-range surface-to-surface missile, has a range of just 250 km. It has yet to enter the production phase.

DRDO would have launched Agni secretly had there been no population near the test range. As it turned out, opposition by some 12,000 villagers to mass evacuation from their homes in the safety zone brought the event under the glare of national and international publicity. Meanwhile, DRDO has not given any indication of when it will fire Agni next.

K.S. Jayaraman

## INTERNATIONAL RESEARCH

# Hitachi opens new labs

## Tokyo

HITACHI Ltd of Japan last week announced the opening of four research laboratories in the United States and Europe that will form close ties with nearby universities. Hitachi's move is the latest in a series of steps by Japan's industrial giants to form links with universities in the West, and it has raised fears that Japan is trying to tap the brains of Western academic institutions. But university researchers involved in the collaboration see the joint research as mutually beneficial.

Hitachi's new laboratories, which will open within the next few months, will be in the Cavendish Laboratory of the University of Cambridge in the United Kingdom, the O'Reilly Institute of Trinity College at the University of Dublin in Ireland, and within Hitachi America Ltd at San Francisco and Detroit in the United States. The arrangement with the University of Cambridge is particularly novel.

The Cavendish Laboratory, one of the world's leading research institutes with a string of Nobel prizewinners to its name, has been desperately seeking funds for a new building for its microelectronic researchers who are currently housed on a separate site at the Science Park in Cambridge. The lease on their building in the park runs out in September and cannot be renewed, according to Dr H. Ahmed, head of the microelectronics laboratory.

Unable to get support from the government, the university has had to dig into its trust funds and turn to Hitachi and another Japanese company for help. Hitachi has donated £250,000, or nearly a third of the £800,000 needed for construction of the building, and Japan's Toshiba Corporation has also given £50,000, says John Deakin, secretary of the Cavendish Laboratory. Construction is expected to be completed in December.

Toshiba's donation is not unusual. Many companies, for example Britain's Rolls-Royce, have contributed to research at the University of Cambridge. What is unusual is that, in return for its large donation, Hitachi will gain control of about a quarter of the new building with its own private entrance.

Hitachi will set up joint research projects with University of Cambridge researchers. The first couple of Hitachi researchers will arrive in May and will collaborate with Ahmed and one of his students on the computer simulation of future microelectronic devices. When the new building opens in December, Hitachi will increase its laboratory staff to about ten, including several British researchers, and the company will pump in annual research funds of about ¥200-250 million (about £1 million), says Hitachi vice president Hiroshi Watanabe. The research

centres in Ireland and the United States will be of similar size. And within about ten years, Watanabe hopes to expand the total number of researchers in Europe and the United States to about 200, dispersed among small laboratories of 10-20 researchers, with most of the staff recruited locally.

Hitachi is just one of several Japanese companies that have recently established research centres linked to universities in the West. Other examples include NEC Corporation, which will open a laboratory in Princeton, New Jersey next month that will carry out joint research on information technology, such as automatic translation machines, with Princeton University. Kobe Steel Ltd opened a polymer composites research laboratory at the University of Surrey in the United Kingdom last October. And Hitachi Chemical will use two-thirds of the space in a new biotechnology laboratory of the University of California in exchange for a \$12 million donation.

Many Japanese companies have for

several years been making donations to leading US universities, for example through the endowment of university chairs. But the move to establish joint research centres is a new phenomenon and there are fears in the West that it is an attempt by Japanese companies to pick the brains of the Western world.

Ahmed, however, sees the collaboration in quite a different light. Japan leads the world in much of the research and development of microelectronics. As well as Hitachi gaining access to the University of Cambridge, Ahmed's student will spend six months in Hitachi's Central Research Laboratory in the outskirts of Tokyo which is equipped with one of the finest clean rooms in the world.

Hitachi's local subsidiary will retain patent rights to all joint research carried out with the University of Cambridge. But the Cavendish Laboratory will get "about 50 per cent" of patent royalties under its agreement with Hitachi, says Deakin. And both Deakin and Ahmed are confident that the university will gain both financially and academically from its collaboration with the Japanese company.

**David Swinbanks**

## MARINE SCIENCE

# Australia to seize the seas?

## Sydney

A NEW report on Australia's marine industries recommends a radical reorganization of the nation's marine science and technology capabilities and calls for Australia to claim its Exclusive Economic Zone, an area bigger than its land mass. But the federal Department of Science appears to be in no hurry to submit to cabinet the report's recommendations, which would cost A\$11.7 million to put into effect. The full report will be made public shortly.

Increasing interest by foreign governments in Australia's fishing zones, the growth in visitors to the Great Barrier Reef and the potential of off-shore oil and gas prompted the government last year to convene a committee chaired by Professor Ken McKinnon, vice-chancellor of Wollongong University.

Marine industries are at present worth more than A\$16,000 million annually, of which A\$4,500 million is gross export income. The federal government spends A\$70 million a year on marine research and development but there has been no growth for four years.

In line with the government's view that industry should help pay for the development of technology, the report recommends upgrading of areas such as aquaculture, marine biotechnology and seabed mineral assessment. "The picture which emerges is that our efforts in the marine field are unbalanced. A large proportion

is concentrated on marine biology and too little in the physical sciences and technology. There are vast amounts of money to be made in marine technology fields, the incentive for industry is untapped profit", McKinnon said.

The report says that previously unharvested species of fish could be exploited in Australia's Exclusive Economic Zone and calls for a personal levy on tourists visiting the Great Barrier Reef "to help maintain the reef in its original state". The committee proposed the formation of a statutory Australian Marine Industries and Sciences Council (AMISC) with responsibility for policy, planning and funding. Eighteen different government departments have an interest in marine sciences, according to the report, so there is likely to be disagreement as to which should take responsibility.

Joe Baker, director of the Australian Institute of Marine Science (AIMS), welcomed the recommendations. "Overall the report brings together data that has been, until now, scattered. We recognize that there must be the incentive for industry to invest in the marine environment and we recognize that, ultimately, AIMS, together with the oceanography and fishing divisions of the Commonwealth Scientific and Industrial Research Organisation and some components of the Bureau of Meteorology and the Antarctic Division will have to be incorporated into a larger structure."

**Tania Ewing**

## Theory versus practice

SIR—As a mere data-generator, I am no doubt ill-equipped to follow the deep theoretical meditations of Virginia A. Huszagh and Juan P. Infante ("The hypothetical way of progress"; *Nature* 338, 109; 1989), far less to criticize them. I am, apparently, caught up in a labyrinth of authoritarian ideology, stale paradigms and pseudo-hypotheses; nor is there anyone who could show me the way out of this maze, as all the creative minds have been driven off, and my fellow-prisoners are mediocre imitators who are unable even to make simple distinctions between speculation and theory. As a wretched product of such a dismal environment, shall I be forgiven for advancing the non-scientific and sociologically based view that this was one of the most inane pieces to be published in *Nature* in many months?

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SIR—Huszagh and Infante make several valid points in their plea for more encouragement for theoretical papers in biology. However, as the editor of a journal (*BioSystems*) which receives (and encourages) theoretical contributions, I take exception to the generalization that journals and reviewers tend to dismiss theoretical work out of hand. As Huszagh and Infante themselves acknowledge, there is much *pseudo*-theorizing, and the overall rejection rate in this area is probably justifiably high. More might be done to stimulate interactions between theoreticians and experimentalists by broadening our overspecialized science curricula, rather than by awarding grants.

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SIR—It is with great interest and an immense sense of relief that we read the Commentary by Huszagh and Infante. The authors echo our own fears and experiences as PhD students. A PhD thesis seems meant solely to be a repository of unwieldy data — and data that conforms with the literature at that.

Things are not very different at seminars. Research data are presented and a particular explanation is offered where two or more possibilities might coexist and they are all dismissed on the grounds that "such examples are not found in the literature". Does the past trend of results govern the future course of science?

It is of course man's ability to think that changes the course of science, not the mere compilation and arrangement of data. Albert Einstein said: "... it appeared that it was possible to get certain knowledge of the objects of experience by means of pure thinking ... Nevertheless, for anyone who experiences it for the first time, it is marvellous enough that man is capable at all to reach such degree of certainty and purity in pure thinking as the Greeks showed for the first time to be possible in geometry".

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## New kidneys

SIR—The leading article (*Nature* 337, 393; 1989) about replacement kidneys reaches a conclusion with which few would disagree, namely that better international arrangements are need for collecting, matching and delivering kidneys to where people need them. In reaching this conclusion, the writer cites two reasons why a commerce in replacement organs should not be unthinkingly banned.

It would be imprudent to ban any practice unthinkingly, but if it is accepted (perhaps naively) that the role of the state is to ensure the well-being of its people, the first reason, as exemplified by the case of a parent who sells an organ in order to provide proper care for a handicapped child, becomes less compelling. An indigent parent is naturally willing enough to do all in his or her power to help a handicapped child, but society should not require that such a parent be exploited by the organ trade. The second reason given is that a law aiming to ban a commerce in replacement organs is unlikely to be effective. Similar objections were probably raised to laws against the slave trade. Admittedly, it is quite possible to draft an ineffective law, but much more frequently it is the enforcement of a law that is ineffective because enforcement depends to a large extent upon political will.

We are then left to consider the morality of a commerce in replacement organs. Commerce implies profit and an indigent who sells an organ is unlikely to profit from the sale. I do not know of any study of the longevity of organ donors, but it is possible that donation may adversely affect the life expectancy of a donor. If loss of life expectancy occurs during the earning period of a vendor's life, the profit from an organ sale that accrues in the long term to a seller is apt to be minimal.

There is reason to believe that a signifi-

cant part of the profit from the sale of a replacement organ ends up not with the person who is the source of that organ but with the entrepreneur who arranges the deal. While the donation of an organ is an act greatly to be admired, reason requires that an exploitive commerce in replacement organs be speedily proscribed by internationally accepted and enforced legislation.

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## Apoptosis or apoptomeatic

SIR—The use of the word 'apoptosis' to describe the active death of a cell is not new. The term is first attributable to a poet, Dioscoriades, who used it in the first century to describe the seasonal falling of flowers<sup>1</sup>. The Greek medical authors Hippocrates and Galen use the word to denote hair loss in balding gentlemen, or the weakening of bones with age. All these events could be caused by an active, programmed cellular response. Greater artistic licence was taken in the second century, with Esais, another Greek poet, using 'apoptosis' to refer to 'falling by the wayside' or 'backsliding' in a theological sense<sup>2</sup>. This is presumably the origin of the definition located by Kleine<sup>3</sup>. Modern English dictionaries do not list the word, but contemporary medical ones do; they dissect it into 'apo' for 'head' and 'ptosis' 'to fall', with particular reference to balding. This may be an artificial division, but the exact origins of the term are unclear.

The 'apoptotic' T lymphocyte is something of a linguistic menagerie. 'Lympho' is derived from 'limpa', a Latin word for 'clear water', the 'p' being corrupted through Greek influence to 'ph' (just as the nypae became the nymphae). 'Cyte' on the other hand is a true Greek word meaning 'hollow vessel'. Clearly the process of activated cell death deserves accurate description: an alternative Greek word is 'apoptomeatic', this being an 'unlucky chance' or 'loss' as used by the authors Polybius<sup>4</sup> and Athaneus<sup>5</sup>. However, as it is not mere fortune but instead a specific antibody that initiates cell death, as described by Smith *et al.*<sup>6</sup>, 'apoptosis' remains the more reasonable alternative. Perhaps 'apoptosis' should join the growing list of scientific terms for review in standard English dictionaries.

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1. Dioscoriades *Prooem* 1, 8.
2. Esais *Parologia Graeca* 34, 4.
3. Kleine, B. *Nature* 337, 402 (1989).
4. Polybius *Historicus* 11, 26.
5. Athaneus *Mathematicus*.
6. Smith, C. A. *et al.* *Nature* 337, 181–184 (1989).



# The making of modern chemistry

George B. Kauffman

Two hundred years ago Lavoisier's *Traité élémentaire de chimie* was published in Paris. The book stands alongside Newton's *Principia* as a landmark work in science.

"CHEMISTRY is a French science. It was founded by Lavoisier of immortal fame". Although many would argue with the French chemist Charles-Adolphe Wurtz's chauvinistic, exaggerated characterization of chemistry, few would dispute his characterization of Antoine-Laurent Lavoisier (1743–1794) as the founder of the subject in its modern form. Lavoisier himself was aware of the revolutionary nature of his work. On February 20, 1773, in opening a new research notebook — the first of his celebrated *registres de laboratoire* — he announced:

Before commencing the long series of experiments that I propose to make on the elastic fluid which is released from bodies, whether by fermentation, or distillation, or finally by every combination, as well as on the air absorbed in the combustion of a great number of substances, I believe that I ought to put some reflections here in writing, in order to shape for myself the plan which I must follow. . . . The importance of the objective impels me to repeat all that to me seems suitable to bring about a revolution in chemistry and physics<sup>1</sup>.

The spread of Lavoisier's revolutionary doctrines was helped by his classic *Traité élémentaire de chimie*<sup>2</sup>, which was published two hundred years ago and represents the culmination of his life's work. When Lavoisier began chemical research, the aristotelian four-element theory was still widely accepted. According to the theory, all substances were composed of four elements — earth, air, fire and water — in varying proportions, and could be transmuted into one another by changing the proportions. In his *Sceptical Chymist* (1661) Robert Boyle had criticized both this theory and the three-principle (mercury, sulphur and salt) theory of the Arabs and Paracelsus; Boyle suggested that an element was a substance that could not be decomposed any further, but his views were not widely held.

Chemistry was first systematically ordered in the late seventeenth and early eighteenth centuries by Georg Ernst Stahl, who developed the phlogiston theory from his teacher Johann Joachim Becher's concept of 'fatty earth', the element of inflammability. Unlike the four-element and three-principle theories, this theory, which held sway until the ideas embodied in the *Traité* were accepted, explained not only chemical composition but also chemical reactions and processes. All combustible substances were considered to contain the hypothetical princi-

ple of phlogiston, the word being derived from the aristotelian term for fire element. Theoretically, phlogiston was released during combustion in the form of fire, flame and sometimes light. The same idea was applied to the process of calcination (or rusting) of metals:

## Phlogiston

Metal – phlogiston = calx

Calx + phlogiston = metal

## Lavoisier

Metal + oxygen = metal oxide  
(oxidation)

Metal oxide – oxygen = metal  
(reduction)

On 1 November 1772 Lavoisier deposited with the Académie des Sciences a sealed note (*pli cacheté*) describing his initial experiments on combustion, the first steps towards his 'revolution in chemistry'. In it he reported that, on burning, sulphur and phosphorus did not lose weight (as might be expected from a loss of phlogiston), but instead increased in weight because they absorbed "a prodigious quantity of air"; while litharge (PbO), in forming lead by reduction with charcoal, did not gain weight (as might be expected from a gain of phlogiston) but decreased in weight because it had lost "air". He did not explain the exact nature of the "airs". It was only after Joseph Priestley's discovery of "dephlogisticated air" (oxygen) in 1774 that Lavoisier realized that in combustion and calcination only a portion of the common air was used up; he decided that Priestley's newly

discovered "air" was what was absorbed ("vital air"), and that "nonvital air" or "azote" (nitrogen) was what remained. Lavoisier concluded that air was a mixture of two different "elastic fluids" — one that supported combustion and respiration (the "salubrious part") and one that did not (*Mém. Acad. R. Sci.* 351–367, 1774). He later called the active part of the air "eminently respirable air", and he demonstrated that "fixed air" (CO<sub>2</sub>) was a compound of charcoal with this air (*ibid.* 520–526; 1775).

In a series of memoirs Lavoisier showed that "eminently respirable air" was contained in phosphoric (H<sub>3</sub>PO<sub>4</sub>), vitriolic (H<sub>2</sub>SO<sub>4</sub>) and nitrous (modern, nitric, HNO<sub>3</sub>) acids, and that it combined with non-metals to form acids. He therefore renamed it *principe acidifiant* or *principe oxygène* from the Greek terms for 'acid' and 'to beget' (*ibid.* 535–547; 1778); this name held even after 1810, when Sir Humphry Davy showed that hydrogen, not oxygen, was the essential constituent of acids. During the winter of 1782–1783, Lavoisier, together with the mathematician Pierre-Simon de Laplace, invented the first ice calorimeter, with which they measured the heat generated in various chemical and physiological reactions, thus establishing the fields of thermochemistry and physiological chemistry. This research led them to conclude that respiration was a kind of combustion. Lavoisier now began to attack the phlogiston theory (*ibid.* 592–600; 1777) and maintained that combustion and calcination were combi-



Lavoisier at work investigating the phenomenon of respiration. The scene was depicted by his wife, a talented woman, who also drew the copperplate illustrations which adorn the *Traité*.

nations with oxygen rather than decompositions (loss of phlogiston).

In 1783 the Englishman Henry Cavendish obtained water by burning "inflammable air" (hydrogen) in common air and in "dephlogisticated air", but he assumed that the water was originally present as a constituent of both "airs". Lavoisier repeated Cavendish's experiment quantitatively, and on 12 November 1783 he made the historic announcement to the Académie des Sciences that water, previously considered one of the four elements, was a compound of "dephlogisticated air" and the aqueous inflammable principle (*principe inflammable aqueux*) (*Observations sur la physique* 23, 452–455; 1783). This work led to the experiments on the combustion of alcohol and other organic compounds in oxygen, which represent the beginnings of quantitative organic analysis.

Lavoisier's new antiphlogistic theory of combustion slowly gained adherents. Beginning in 1782 Louis Bernard Guyton de Morveau, Claude Louis Berthollet, Antoine François de Fourcroy and Lavoisier collaborated in devising a new system of nomenclature, based on the new chemistry, which led in 1787 to the *Méthode de nomenclature chimique*<sup>1</sup>. This influential book listed provisionally as elements 55 substances that had not been decomposed (*substances non décomposées*). The system marked a complete break with the past and replaced the old fanciful names such as butter of antimony, oil of tartar, spirit of hartshorn and so on by new ones that corresponded to their chemical composition. With some modifications, the new names form the basis for our modern system of nomenclature.

The *Traité élémentaire de chimie* grew out of the *Méthode* and was published in March 1789. It was neither a general reference work nor a technical monograph, but a book for beginners which summarized the discoveries of Lavoisier and his co-workers and which introduced the new chemistry. In the preface Lavoisier described his pedagogical ideas on the learning of science, the new nomenclature and his reliance on experiment:

We must trust to nothing but facts: These are presented to us by Nature, and cannot deceive. We ought, in every instance, to submit our reasoning to the test of experiment, and never to search for truth but by the natural road of experiment and observation.

He rejected the four aristotelian elements and the three paracelsian principles, and he admitted the provisional nature of his own table of elements, which he called *substances simples*:

We cannot be certain that what we regard today as simple is really so; all that we can say is that such a substance is the actual limit reached by chemical analysis, and that, in the present state of our knowledge, it cannot be further subdivided.

The first edition of the *Traité* was published in two volumes, continuously paginated and divided into three parts. In Lavoisier's own words:

In the first part of my work, I make very little use of any experiments but those which were made by myself. . . . The second part is composed chiefly of tables of the nomenclature of the neutral salts. To these I have only added general explanations, the object of which was to point out the most simple processes for obtaining the different kinds of known acids. This part contains nothing which I can call my own, and presents only a very short abridgement of the results of these processes, extracted from the works of different authors. . . . In the third part, I have given a description, in detail, of all the operations connected with modern chemistry . . . this part could not be borrowed

TABLEAU DES SUBSTANCES SIMPLES.

	NOMS NOUVEAUX.	NOMS ANCIENS CORRESPONDANTS.
	Lumière. . . . .	Lumière.
	Calorique. . . . .	Chaleur.
		Principe de la chaleur.
		Fluide igné.
		Feu.
		Matière du feu et de la chaleur.
		Air déphlogistiqué.
		Air empyrémat.
		Air vital.
		Base de l'air vital.
		Gas phlogistiqué.
		Métalle.
		Base de la mofette.
		Gas inflammable.
		Base du gas inflammable.
		Soufre.
		Phosphore.
		Carbone.
		Charbon pur.
		Inconnu.
		Inconnu.
		Inconnu.
		Antimoine.
		Argent.
		Arsenic.
		Bismuth.
		Cobalt.
		Cuivre.
		Étain.
		Fer.
		Manganèse.
		Mercure.
		Molybdène.
		Nickel.
		Or.
		Platine.
		Plomb.
		Tungstène.
		Zinc.
		Chaux.
		Terre calcaire, chaux.
		Magnésie, base de sel d'Épau.
		Barote, terre pesante.
		Argile, terre de l'alun, base de l'alun.
		Alumine.
		Silice.
		Terre siliceuse, terre vitifiable.

The table of elements published in the *Traité*, here reproduced from the reprint of 1864.

from any other work, and. . . in the principal articles it contains, I could not derive assistance from anything but the experiments which I have made myself.

In Chapter XIII (Part I) Lavoisier reported a hitherto unpublished quantitative experiment, stating most clearly the law of conservation of matter during chemical reactions:

Nothing is created either in the operations of the laboratory, or in those of nature, and one can affirm as an axiom that, in every operation, there is an equal quantity of matter before and after the operation; that the quality and quantity of the principles are the same, and that there are only alterations and modifications. On this axiom is founded the whole art of making experiments in chemistry: we must suppose in all of them a true equality or equation between the principles of the body one examines, and those that we extract by analysis.

Actually, the idea that matter is neither created nor destroyed originated with the Greek atomists and was commonly assumed by eighteenth-century scientists.

Lavoisier, however, was the first to apply it specifically to chemical reactions. The historian of chemistry, John Riddick Partington, also considered this the first use of the word 'equation' in the sense of chemical equation. In Lavoisier's words, "must of grapes = carbonic acid + alcohol". Ironically, in this case Lavoisier's data contained considerable errors, yet they largely cancelled each other out and yielded a result close to equality.

Part II, the shortest part, includes a "Table of Simple Substances", a modified version of the *Méthode de nomenclature chimique* and the first modern list of the chemical elements. Part III, almost half of the book, deals with a wide variety of techniques and apparatus. Altogether the *Traité* is a truly modern work — in Douglas McKie's words, "Lavoisier had done for chemistry what Newton had done for mechanics a century earlier in his *Principia*".

As a political liberal who sympathized with many of the criticisms against the *ancien régime*, Lavoisier took part in the events leading to the French Revolution. Nevertheless, he was attacked by the radical politician and journalist Jean-Paul Marat. In 1768, Lavoisier had become a member of the Ferme Générale, the government's primary tax-collecting agency, and during the Reign of Terror, the Revolutionary Convention passed a decree on 24 November 1793 ordering the arrest of the former members of the agency (which had been abolished in 1791). On the morning of 8 May 1794, together with 27 of his former colleagues, including his father-in-law, Lavoisier was tried and convicted by the Revolutionary Tribunal. All were guillotined that very afternoon. The widely circulated remark, "The Republic has no need of scientists; let Justice take its course!", attributed to Jean Coffinhal, president of the Tribunal, is apocryphal. But the comment made the following day by Joseph Louis Lagrange is authentic — "It took them only an instant to cut off that head, and a hundred years may not produce another like it". □

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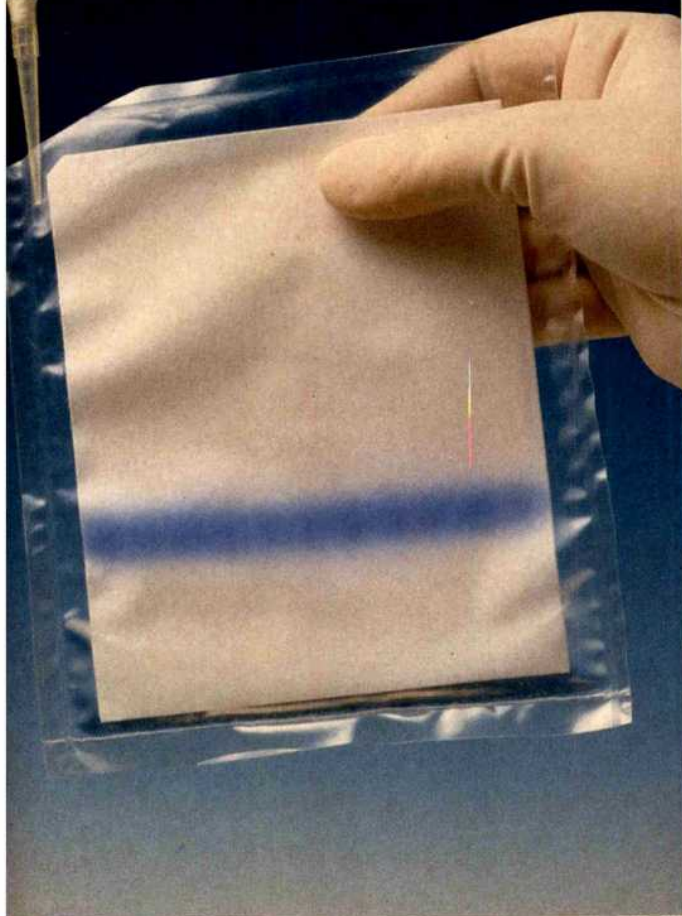
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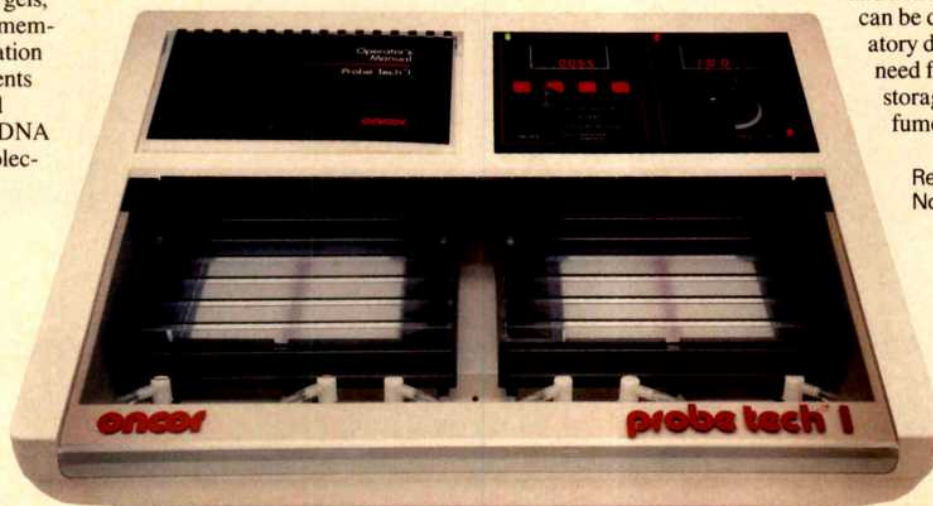
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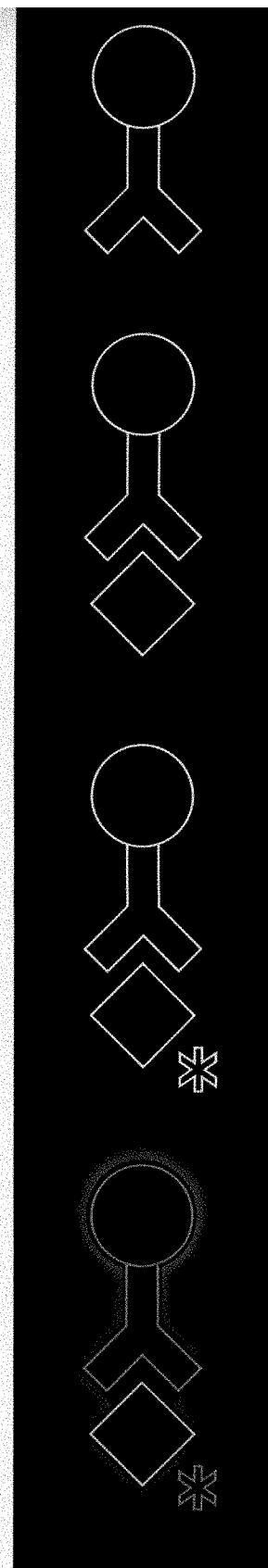
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# What to say about cold fusion

Public interest in recent excitements is to be welcomed, especially if it does not turn to anger when attempts to replicate the observation of cold fusion fail — the most probable outcome.

FLEISCHMANN and Pons have done at least one great service for the common cause: they have kindled public curiosity in science to a degree unknown since the Apollo landings on the Moon. In the past few weeks, people thought by those about them to have a few scraps of inside knowledge have been quizzed about the smallest details of the experiments at the University of Utah — those first described in the *Wall Street Journal* and the *Financial Times* on 23 March and at a press conference at Salt Lake City later that same day. The social phenomenon is all the more remarkable because of the wide distribution of those who have a general understanding of nuclear fusion and of the reasons why its practical application would be important.

These impromptu conversations seem generally to be remarkably good-humoured. Those who want to know more, and who in particular ask "Do you think it's true?", seem not too much annoyed by the prospect of continued suspense. It is remarkable that so many people are willing to accept that experimental observations, and the inferences drawn from them, acquire validity only by replication. Has what used to be called "the scientific method" now become widely understood?

If so, much of the credit should go to the daily press, which has risen superbly to the challenge of cold fusion. The two financial newspapers that broke the news on 23 March did so in cautious language, making it plain that cold fusion was not then a proven reality, let alone a commercial source of limitless energy. Other daily newspapers have joined in with commendable zeal and sobriety. Inevitably, the US daily press, with its resources and self-discipline, has done best, but newspapers such as the London *Daily Telegraph*, with no great tradition in the field of science reporting, have been magnificent. Reporters have given full and coherent accounts of meetings as far apart as Sicily and Texas, have canvassed opinions with care and have also faithfully reflected the good humour of these hectic weeks.

The good humour may not persist if the experiments cannot be replicated but there are some grounds for optimism. Part of the reason why so many people are so interested seems to be the general delight that a couple of people in widely separated universities have used their own money to pull off a trick on which governments have

lavished huge sums of money in the past 30 years, so far without result. So people may well be indulgent if the attempts to replicate cold fusion on the scale described by Fleischmann and Pons prove failures. "It was a brave venture", they may say, "What a pity it did not succeed!"

That would be the best outcome. It is also, of course, possible that the general reaction to the failure of attempts at replication will be more sour. The scientific community's reputation is vulnerable in several respects, not the least of which is that neither the Utah group nor the Brigham Young group (whose account of its work appears on page 737, this issue) had, before seeking publication, carried out the rudimentary control experiment of running their electrolytic cells with ordinary rather than heavy water.

A close friend who is a Soviet biologist, on the telephone from the United States last week, was indignant at this neglect. He is correct. How is this astonishing oversight to be explained to students repeatedly being drilled in the need that control experiments should be as conspicuous in the design of an investigation as those believed to display the phenomenon under study? And how should the neglect be explained to the world at large?

There is no convincing explanation, only extenuating circumstances. Self-imposed secrecy has evidently hampered the investigators, understandably buoyed up by their belief that they had discovered a remarkable new phenomenon and fearful that too much talk about it would give other bigger battalions a chance to steal a march on them. Yet it is unthinkable that, if the authors had felt able from the outset to stand in front of routine laboratory colloquia and give a full account of their work, the question "Have you tried it with ordinary water?" would not have been raised. This glaring lapse from accepted practice is another casualty of people's need to be first with reports of discovery and with the patents that follow.

More subtle doubts perplexing those seeking to replicate the experiments would have been exorcised in the same process. The Brigham Young group, for example, clearly explains how it has been necessary to estimate the number of neutrons emitted from its electrolytic cells by subtracting from its observations of neutrons the background measurements, but Dr John M. Carpenter (one of the referees of the article by Jones *et al.*)

explains on page 711 that the background may fluctuate with time, which argues for the need for contemporaneous controls. Sadly, the neutron monitor is a special device, of which there is only one copy....

The Fleischmann and Pons experiments raise bigger questions, if only because the scale of the phenomenon they report is so much greater. Both groups report the detection of neutrons, but the Utah group (*J. Electroanal. Chem.* **261**, 301; 1989) requires that there should be  $10^{12}$  fusion reactions a second (within an order of magnitude in either direction) to account for the rate at which heat is produced, while the Brigham Young group is talking of one fusion reaction every 100 seconds or so. But the Utah group has not yet dealt with the natural question whether the observed energy output is energy stored in the palladium electrodes during the preparation of the cell. Records of cell voltage and current for the full duration of each run would go some way to settle the question, but have not been produced.

So are the extenuating circumstances sufficient to avoid the conviction of the scientific community for irresponsibility? No doubt the general opinion will depend on the outcome of attempts at replication, but the community might wish that its reputation did not hang on such a narrow thread, especially because the likelihood of replication fades as the days go by.

So robust scepticism is the only wise view. There may be something in the Brigham Young phenomenon, but that requires careful confirmation. The Utah phenomenon is literally unsupported by the evidence, could be an artefact and, given its improbability, is most likely to be one.

Luckily, there are a few blessings to count. Theoreticians have zealously recalculated the fusion rate between deuterons in a molecule (now reduced by 10 orders of magnitude) and have shown that the fusion rate between protons and deuterons, against naive expectation, is likely to be six orders of magnitude greater still — but still no more than  $10^{-14}$  per molecule of HD per second. A week or so ago, ingenious schemes for bringing bare hydrogen nuclei more closely together in the electron sea of a palladium lattice seemed to offer an escape from scepticism. But not for long, even if it is plain that metal hydrides are an even more interesting field of research than had been thought.

John Maddox

# The descent of the larynx?

John C. Marshall

AMONG the uninitiated the discovery of an old human hyoid bone reported by Arensburg *et al.* on page 758–760 of this issue<sup>1</sup> is likely to raise three questions: what is it; where is it; and why should anyone care? The answer to the first question is a small U-shaped bone, consisting of a body with two greater and two lesser horns. Between the root of the tongue and the larynx is the answer to the

It is at this point that the hyoid bone makes contact with a long-running controversy about the evolution of speech. Extant primates (monkeys and apes) do not spontaneously produce the range of phonetic vocalizations characteristic of human speech, and attempts to teach them to do so have met with very limited success<sup>2</sup>. Does their 'failure' reflect differences in the anatomical configuration of

their vocal tracts, or is it rather the case that they lack the requisite neuronal structures for the control of articulate speech? Computer-implemented modelling of the supralaryngeal vocal tract seems to indicate that some, but not all, vowels and consonants are "virtually impossible for the chimpanzee to articulate" (ref. 3).

These simulations by Lieberman, Crelin and Klatt<sup>3</sup> have not, however, met with unqualified acceptance. For example, the point vowels *i*, *u* and *a* cannot be simulated by their reconstruction of the chimpanzee vocal tract,

what degenerate specimen, deformed in life by arthritis, and after death by the very process of fossilization. Moreover, his hyoid bone is missing. As Lieberman<sup>5</sup> himself writes in a later discussion of the problems that arise when one tries to infer speech and language capacities from a fossil: "Nothing remains of the brain, the soft tissue of the vocal tract, or small bones like the hyoid and the cartilages of the larynx".

One of these statements has now been falsified. Arensburg *et al.*<sup>1</sup> have recovered a well-preserved human hyoid bone from Middle Palaeolithic layers of Kebara Cave, Mount Carmel, Israel. The specimen dates from approximately 60,000 years BC. Furthermore, the size and shape of this hyoid, and the positioning of the marks left by muscle attachment, are, for the most part, within the range of modern man. It is well known that the middle ear ossicles of Palaeolithic Neanderthals are likewise very similar to our own. Arensburg *et al.*<sup>1</sup> accordingly hypothesize that "the osseous elements derived from the embryonic branchial arches show less rapid evolutionary changes than bones derived from dermal or enchondral tissues". And they boldly speculate that the larynx itself may not have altered significantly since the Middle Palaeolithic.

Their evidence about the hyoid certainly makes it more likely that Neanderthal man was morphologically adapted to speech, although they do not elaborate in their paper on the hypothetical positioning of either the hyoid or the larynx. The inference that the Neanderthal larynx was positioned too high in the throat to produce *i*, *u* and *a* was a crucial feature of Lieberman's model<sup>3</sup>. It is generally accepted that the larynx descended in two stages and that in the first stage only neutral vowels could be formed. Consistent with this claim, Kratz<sup>6</sup> reports that

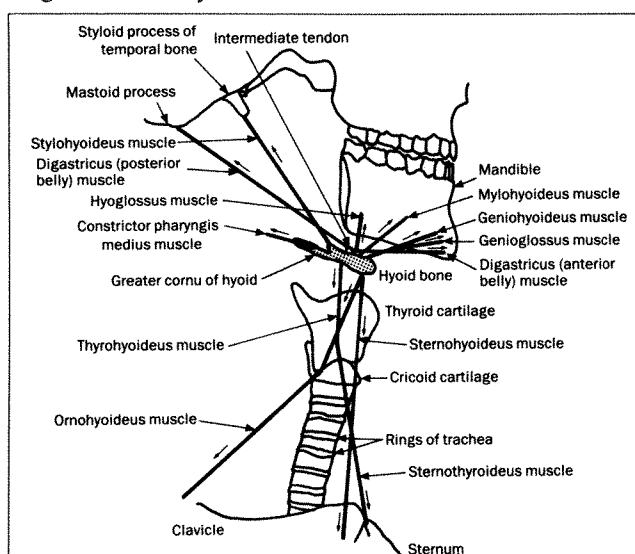


FIG. 1 The extrinsic laryngeal musculature showing the direction of movement of the hyoid bone, when the muscles contract from fixed origins. (From ref. 12.)

second. The third question is, of course, the interesting one.

In addition to supporting the base of the tongue, the hyoid bone is connected to the cartilaginous framework of the larynx by eleven muscles. Accordingly, any movement of the hyoid affects the positioning and movement of the larynx (Fig. 1). The extrinsic muscles of the larynx form two groups; the suprahyoid muscles, which act as laryngeal elevators; and the infrahyoid, which act as laryngeal depressors. These changes in turn affect the configuration of the supralaryngeal vocal tract and hence the nature of the noises produced when quasi-periodic bursts of air from the larynx are released through the oral and nasal cavities. Thus vigorous raising of the larynx decreases the volume of the supra-glottal cavity and thereby increases the supra-glottal pressure, to an extent permitting the production of such ejective sounds as *p'* or *t'*. Similarly, when the anterior belly of the digastricus muscle contracts from a fixed mandible (lower jaw), the hyoid, and hence the tongue, will move anteriorly and upward. The movement is required for such alveolar articulations as *s* or *i*. In short, then, the humble hyoid is vitally implicated in the mechanics of human speech production.

yet Jordan<sup>4</sup> reports that (real, live) chimpanzees do produce at least two of them! Lieberman and colleagues also claim, on the basis of a reconstruction of the La Chapelle-aux-Saints fossil, that Neanderthal man could not produce the point vowels. This skull, however, is a some-



FIG. 2 Conversation piece? An artist's impression of a Neanderthal family, by Maurice Wilson.

## HIGH-PRESSURE MINERALOGY

## Mechanisms of phase changes

D. C. Rubie

"linguistic reconstruction of the preproto-Indo-European language indicates an absence of differentiated vowels", although he dates this prehistoric language from a mere 10,000 years ago.

With respect, however, to the fossil record, it must be stressed that the distance from morphology to function is considerable. Who would have guessed solely from consideration of the anatomy of the syrinx — a structure very different from the larynx — that the mynah bird could produce such a passable imitation of human speech? Who, for that matter, would suspect that humans can learn to produce intelligible speech after surgical removal of the tongue, or of the larynx? Severe deformity of the vocal tract is compatible with human speech if a human brain is in command thereof. There are some lateral asymmetries of the modern brain that leave an impression on the skull, and these can be found on endocasts of Neanderthal man<sup>10</sup>. Such asymmetries at the posterior end of the sylvian fissure are thought to be related to linguistic capacity, although they can also be seen in apes that most emphatically do not possess language abilities.

The best witness to Neanderthals' intellectual level can perhaps be found in reconstructions of their culture (Fig. 2). There is good evidence of advanced stone tool manufacture, and presumptive evidence that Neanderthals "buried their dead with symbolic artifacts, including red ochre, animal bones, and flowers"<sup>11</sup>. The possibility that Neanderthals engaged in ritual murder<sup>11</sup> also links them to modern man, although whether one wants to regard this as an example of 'high culture' is, I suppose, debatable. All our inferences about the language, thought and culture of Neanderthals are based upon long, fragile and indirect lines of reasoning. The argument about their language capacity will undoubtedly run and run until we discover a deep-frozen Neanderthal who is susceptible to resuscitation. □

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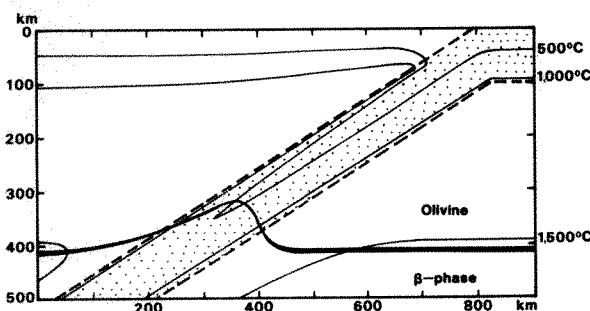
THE most abundant phase in the Earth's upper mantle is orthorhombic (Mg,Fe)<sub>2</sub>SiO<sub>4</sub> olivine. With increasing pressure, this phase transforms sequentially to the higher-density polymorphs  $\beta$ -phase (modified spinel structure) and  $\gamma$ -spinel. The transformation of olivine to  $\beta$ -phase coincides with and is generally believed to be responsible for the seismic discontinuity observed at a depth of 400 km. The mechanism of this phase transition, which is important in discussions of subduction dynamics, mechanisms of deep-focus earthquakes and the rheology of subducting lithosphere, has been a subject of debate for over 10 years. The new results of Burnley and Green reported on page 753 of this issue<sup>1</sup> now show without doubt that olivine can transform to the high-pressure spinel structure by two different reaction mechanisms, depending on the magnitude of differential stress as well as

mantle (see figure). The density of  $\beta$ -phase is about 8 per cent greater than that of olivine, and thus an elevated phase boundary such as that shown in the figure would produce a large negative buoyancy force which would contribute significantly to the driving forces of subduction and mantle convection<sup>2,3</sup>.

But if temperatures in the subducting slab are as low as suggested by thermal modelling (500–600 °C at a depth of 300 km), the kinetics of the transformation may become important in determining the position of the phase boundary. In this case, olivine persists metastably in a subducting slab, the elevation of the phase boundary and the negative buoyancy force are less than that predicted by an equilibrium model, and the rate of subduction may be controlled to a large extent by the kinetics of the transformation<sup>4,5</sup>. For this hypothesis to be

tested, experimental kinetic data must be extrapolated to subduction-zone conditions — a virtually impossible task unless the mechanism of transformation is understood so that an appropriate rate law can be used.

In experimental investigations of the transformation mechanism, compounds such as Mg<sub>2</sub>GeO<sub>4</sub>, Ni<sub>2</sub>SiO<sub>4</sub> and Co<sub>2</sub>SiO<sub>4</sub> have often been used because their high-pressure polymorphs are formed at pressures



Thermal structure of a subduction zone based on numerical modelling. The subducting slab (stippled) is bounded by dashed lines and 500 °C, 1,000 °C and 1,500 °C isotherms are shown (solid lines). The depression of the 500 °C isotherm to a depth of about 350 km in the slab causes the olivine- $\beta$ -phase equilibrium boundary (thick line) to be elevated in the slab ~ 100 km relative to the boundary in the adjacent mantle. (After Helffrich *et al.*<sup>10</sup>.)

the pressure-temperature conditions.

The nature of the transformation mechanism is particularly important in discussions of the driving forces of plate tectonics. Oceanic plates are created at mid-ocean ridges by the process of sea-floor spreading. The complimentary process of plate consumption occurs at ocean trenches where two plates collide and one of the plates descends deep into the mantle by a process known as subduction. The driving force for subduction comes from the density contrast between the cold slab and the hot surrounding mantle (see figure). In addition, a large driving force may result from density increases produced by high-pressure phase transitions such as olivine to  $\beta$ -phase or spinel. The pressure-temperature slope ( $dP/dT$ ) of the olivine- $\beta$ -phase equilibrium boundary is positive so that in subduction zones the position of the equilibrium boundary between the two phases is elevated by as much as 100 km relative to the adjacent

mantle which are readily attainable experimentally (1–6 gigapascal). Phases with these compositions are regarded as analogues of the mantle phases, although only spinel (and not  $\beta$ -phase) occurs as a high-pressure polymorph in Mg<sub>2</sub>GeO<sub>4</sub> and Ni<sub>2</sub>SiO<sub>4</sub>.

One mechanism for the transformation of olivine to spinel or  $\beta$ -phase involves incoherent nucleation (on olivine grain boundaries for example), with subsequent growth controlled by diffusion across the interphase boundary<sup>4</sup>. Another, which is analogous to a martensitic transformation and results from the propagation of partial dislocations, operates by shear of the oxygen sublattice and accompanying 'synchroshear' of the cations<sup>6</sup>. The new results of Burnley and Green<sup>1</sup> on the transformation of olivine to spinel in Mg<sub>2</sub>GeO<sub>4</sub> show that either of these mechanisms is possible depending on the experimental conditions. The incoherent nucleation and growth mechanism operates at low differential stress, high temperatures (above

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700 °C) and conditions relatively close to equilibrium. In contrast, nucleation occurs by the shear mechanism under conditions of high differential stress, moderate temperature (600 °C) and pressure-temperature conditions which deviate significantly from equilibrium.

The different mechanisms observed in earlier experimental studies can now be readily explained on the basis of these new results<sup>1</sup>. Evidence for the incoherent nucleation and growth mechanism has come primarily from experiments carried out in large-volume high-pressure apparatus (for example, piston-cylinder and multi-anvil devices), whereas evidence for the shear mechanism has previously been found only in the products of diamond-anvil-cell experiments. The variability of these previous results can therefore be attributed primarily to the level of differential stress in the high-pressure apparatus.

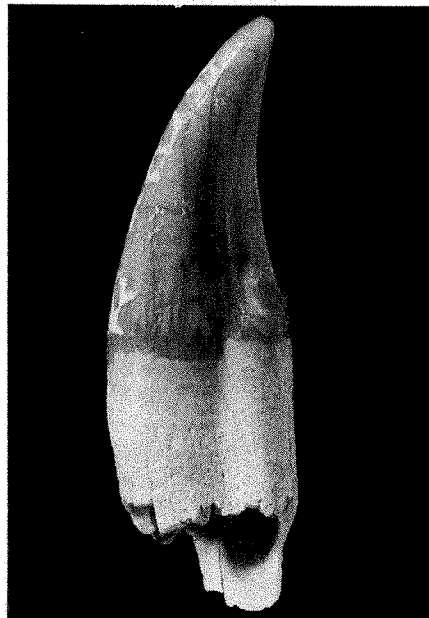
The mechanism of the transformation of olivine to  $\beta$ -phase or spinel both in the mantle and in shocked meteorites can now be considered. At the 400-km discontinuity, where the temperature is high (1,400–1,500 °C) and differential stresses are low, the transformation probably occurs by the incoherent nucleation and growth mechanism. In shocked meteorites, however, olivine is most likely to transform to the high-pressure polymorphs by the shear mechanism under conditions of low temperature and very high differential stress. In subduction zones, where both temperature and differential stress have intermediate values, it is still uncertain which mechanism dominates. Apart from the questions of kinetics and the possible elevation of the phase boundary, there are potentially important implications associated with the two transformation mechanisms.

First the mechanism will influence the post-transformation microstructure and consequently may affect the rheology and mechanical properties of subducting lithosphere. At conditions which deviate significantly from equilibrium, transformation by the incoherent nucleation and growth mechanism results in very fine-grained spinel<sup>7</sup> (grain size 1–3  $\mu\text{m}$ ). In contrast, preliminary results suggest that when nucleation occurs by the shear mechanism, subsequent growth results in much coarser-grained spinel<sup>1</sup>. In the former case, the slab may deform by a grain-size-dependent superplastic mechanism and consequently the strength of the slab would be much lower than in the latter case<sup>8</sup>.

Second, there may be a close relationship between high-pressure phase transformations in subduction zones and the mechanisms of deep-focus earthquakes. Although this idea was first proposed more than 40 years ago, its validity and the nature of such a relationship are both

## Fossils from the Miocene of Abu Dhabi

THE Arabian peninsula was a unique land mass through which faunal elements from Europe, Asia and Africa could migrate and mix in the late Tertiary, about 25–2 million years ago. Its fauna at any particular epoch can be used to infer its geographical connections with other parts of the world. Therein lies the importance of a palaeontological expedition to the United Arab Emirates in January which unearthed an important fauna from the Late Miocene (about 8 million years ago). The fauna,



British Museum (Natural History)

Canine tooth (about 26 mm high) of a macaque-like monkey found in Abu Dhabi.

discovered by Peter Whybrow of the British Museum (Natural History) and his colleagues, consisted of deinotheres (proboscideans distantly related to modern elephants), hippopotami, small carnivores, horses, rhinoceroses, turtles, crocodilians, fish, birds and a macaque-like monkey (see figure). Ostrich eggshells twice as thick as any known today revive the myth of Sinbad's roc, a mythical bird large enough to carry off full-grown bull elephants. The fauna is clearly of Ethiopian origin, but artiodactyl (possibly cervid) limb bones may betray the presence of a few Holarctic elements from south-west Asia.

By 8 million years ago, the Tethys Ocean that had stretched across the Near and Middle East from the Mediterranean Sea to the Indian Ocean had long since closed: this

great seaway had been the overriding factor in animal migrations across the Old World since the late Palaeozoic, when it formed the boundary between the ancient land-masses of Laurasia (North America and Eurasia) and Gondwanaland (the southern continents and India). The Arabian Peninsula became an important land bridge: in the Miocene, the Red Sea was open to the Mediterranean, but was closed at its southern end by a land bridge between Ethiopia and the Yemen. Further east, the Tigris-Euphrates river system extended much further south than it does today. The new fauna represents a thriving community from what seems to have been the delta of this system. This idea is supported by the extent of the fauna, known from sites over an area of 16,000 square kilometres.

Apart from the intrinsic value of the fossils, Whybrow's team was able to refine knowledge of the stratigraphy of the Gulf Neogene. One example of this is their discovery of the horse-like *Hipparion* in the sediments, restricting rocks previously designated as 'undifferentiated Tertiary' to an age of less than 11.2 million years. *Hipparion* is not known from the Old World any earlier than this, and is an important zone fossil.

Well-watered palaeoenvironments from this region may seem surprising in the light of conventional wisdom about the Gulf area, long thought to have been arid for an extended period. The discovery of fossil fish in sandstone structures thought to represent dune fields forced the reevaluation of these formations as fluvial. In fact, desert conditions are unknown from the Miocene of this part of the world. The subsequent desiccation of the peninsula (during the Plio-Pleistocene) is related to increasing aridity worldwide: savannah opened up in Africa and the shrinkage of forests led to the development of ground-living hominids as well as modern plains animals such as grazing horses and ruminants. But the great drying was punctuated by short, wet episodes. In the Pleistocene, these may have been in synchrony with the glacial-interglacial cycle of the northern ice ages. Even 8,000 years ago, large parts of Arabia were green and pleasant, and areas now considered barren produced crops well into historical times.

Henry Gee

still uncertain<sup>9</sup>. The knowledge of the mechanisms of phase transformations in subduction zones should alleviate this uncertainty. □

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## NUCLEAR PHYSICS

# The cold fusion family

James S. Cohen and John D. Davies

CLAIMS for the observation of cold fusion are based on two types of evidence. Jones *et al.*, on page 737 of this issue<sup>1</sup>, report that they have detected neutrons, with energy characteristic of a fusion reaction, in an electrochemical cell containing deuterium. And Pons and Fleischmann report in the 10 April issue of the *Journal of Electro-analytical Chemistry*<sup>2</sup> the production of unexplained excess heat and also neutrons in a similar cell. It is wise to see whether the observations can be explained using standard physics before seeking novel solutions — especially as the two reports conflict greatly in detail.

Cold fusion is a known process, but not as described in the new work. A muon — a heavy analogue of the electron — substituted for an electron in a deuterium molecule can cause the molecule to contract so that there is a significant probability of the two nuclei coming into contact. A fusion reaction can follow in which energy is carried away by the products. The muon is released and can induce further fusions. This is muon-catalysed fusion<sup>3</sup>.

Considerations of this process in the context of the present experiments is natural, because Van Sicken and Jones have previously suggested<sup>4</sup> that very high pressures could similarly induce fusion; because some have suggested that muons from cosmic rays could be responsible for the observed effects; and because lessons about the relative rates of different fusion reactions could be important. The reactions of interest are those of deuterons ( $d = {}^2\text{H}$ ), tritons ( $t = {}^3\text{H}$ ) and protons ( $p = {}^1\text{H}$ ).

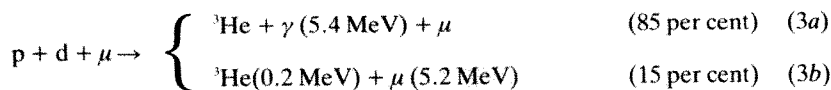
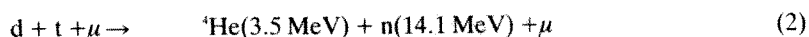
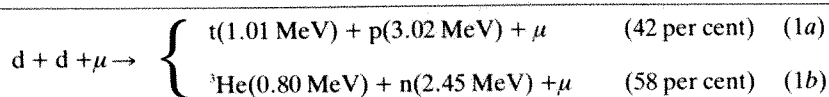
correspondingly. The mean time for a fusion reaction then becomes (ref. 5)  $8 \times 10^{-13}$  s for d-t,  $2 \times 10^{-9}$  s for d-d. (The lesser speed of the d-d reaction arises in part because fusion occurs in a state with one unit of angular momentum which creates a centrifugal barrier to fusion. The d-t molecule is also initially formed in such a state but, because of its asymmetry, rapidly makes a transition to a zero-angular-momentum state.)

It is useful to consider the description of fusion in nuclear-scattering experiments. Besides the probability of barrier penetration, the subsequent interaction between the nuclei (expressed in the 'astrophysical'  $S$  function) has an important influence on the fusion rate or cross-section  $\sigma$ :

$$\sigma = \frac{S}{E} \exp(-31.3 \sqrt{\mu/E})$$

where  $\mu$  is the 'reduced' mass in atomic mass units of the colliding nuclei and  $E$  is the centre-of-mass collision energy in keV. The variation of  $S$  for the various reacting nuclei can be crucial. In the low-energy limit, which clearly applies to cold fusion,  $S = S_0$  and is smallest for the p-d reaction ( $2.5 \times 10^{-4}$  keV barns)<sup>6</sup>; this is because the final stage involves the emission of a  $\gamma$ -ray, an electromagnetic process which is inherently weaker than the strong-force interactions of the other fusion reactions. For d-d,  $S_0$  is  $1.08 \times 10^2$  (including both channels<sup>7</sup>); for d-t, which involves a strong resonant intermediate state of  ${}^3\text{He}$ ,  $S_0$  is  $1.15 \times 10^4$  (ref. 8).

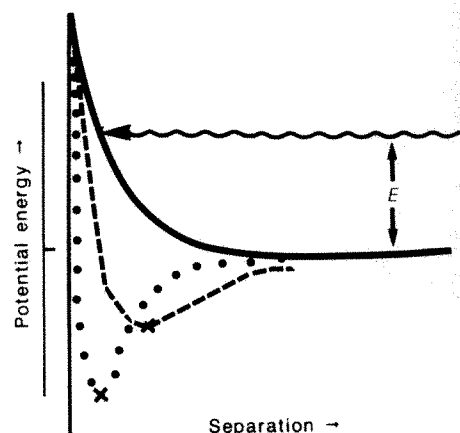
Nevertheless, at low energy the



Here  $n$  signifies neutrons;  $\gamma$ ,  $\gamma$ -rays;  $\mu$ , negative muons; the energy carried by fragments is given in megaelectron volts; and the percentages signify branching fractions in muon-catalysed reactions.

Before fusion, the nuclei are held apart by Coulomb repulsion. This barrier is penetrated by quantum-mechanical tunnelling, rather than being surmounted. Note that tunnelling is controlled more by the width of the barrier than by its height. Thus, replacing the electron in a hydrogen ion by a muon reduces the separation of the nuclei by a factor almost equal to the muon/electron mass ratio (207), reducing the 'tail' of the Coulomb barrier

'Gamow' penetrability factor becomes dominant, and significant variations arise between the various fusion rates because of the variation in reduced mass. Thus for bare nuclei, the d-t fusion rate exceeds that of d-d for collision energies above about 400 eV, and the p-d rate exceeds that of d-d below about 200 eV. The implications of the two factors for the case of cold fusion, in which the Coulomb repulsion is shielded by electron or muon screening, is also instructive. Only the barrier penetrability factor is changed by the screening (see figure); also we have just established some useful energies at which the relative fusion rates change.



Schematic of the potentials between two nuclei (not to scale): solid line, unscreened; broken, with electron screening (chemical bonding); dotted, with muon screening. To the right of the barrier, electrostatic interactions are dominant; to the far left, strong nuclear interactions occur as expressed by the astrophysical  $S$  factor. Wavy line indicates collision energy  $E$  in scattering experiments, the classical distance of closest approach ( $R_0$ ) at the arrow head. The bond length ( $x$ ) is actually about 207 times shorter for muons than for electrons and the potential well is about 207 times deeper.

From these it is possible to show that muon screening favours d-t fusion; normal electron screening favours p-d fusion (although in this case fusion rates are unmeasurably slow).

The characteristic separation in the isotopic hydrogen molecules is the bond length — normally somewhat greater than the distance  $R_0$  at which repulsion between the nuclei becomes apparent. Allowing the nuclei easily to approach this close is roughly equivalent to colliding the bare nuclei together with a centre-of-mass energy  $14/R_0$  eV where  $R_0$  is measured in ångströms. (Such simple considerations have to do for now since an accurate calculation of the penetrability requires knowledge of the potential energy interaction of d-d in the crystal, which is unknown for electrochemical cold fusion.) For the ordinary hydrogen molecule,  $R_0 = 0.7$  Å, so that the equivalent energy is about 20 eV, well below the value 400 eV at which d-t fusion becomes faster than d-d. But with the ion's electron substituted by a muon,  $R_0 = 4 \times 10^{-3}$  Å, so that the equivalent energy is about 3,500 eV and d-t fusion is clearly favoured (by a factor of 36 in the zero-angular-momentum state according to calculations of C.-Y. Hu (personal communication)). Although muon-catalysed fusion presents the most dramatic evidence of the effect of screening on fusion rates, similar electron-screening effects have been seen in reactions such as (ref. 9)  ${}^3\text{He} + d \rightarrow {}^4\text{He} + p$ .

Screening effects should be considered in discussing the new results. Any means of reducing  $R_0$ , vibrational excitation of the bond, a reduction in the bond length, or even a flattening of the potential-

energy well could be effective. It is interesting to note that for the shielding distance relevant to the new studies (of the order of  $10^{-1}$  Å) the p-d fusion rate should exceed that for d-d. This reaction should be studied experimentally; there seems to be no advantage in using d-t, however.

Some have suggested that the new results could be an artefact due to fusion catalysed by cosmic-ray muons. This seems most improbable: the muon stopping flux is not high; the fraction captured by deuterium is low; and the fraction retained by deuterium is even lower. Also, because the muonic molecule formation rate is too slow and because a significant fraction of the muons stick to the helium at fusion, each one can catalyse only a few reactions.

It is reasonable to take  $0.1 \text{ g}^{-1} \text{ h}^{-1}$  to be the upper limit to the rate for muons being stopped in a Utah basement (J. Osborne, personal communication). Heavy elements are better at stopping muons, the capture ratio being roughly proportional to nuclear charge  $Z$  (ref. 10). Moreover, any  $d\mu$  would initially have about an electron volt of kinetic energy<sup>11</sup>: while slowing down, many muons would be transferred to the high- $Z$  atoms<sup>10</sup> (high- $Z$  contaminants are kept to less than  $10^{-4}$  of contents in muon-catalysed fusion experiments). Lastly, the time for formation of the muonic deuterium molecule is comparable to the muon lifetime ( $2.16 \mu\text{s}$ ) so that few fusions can be catalysed by each muon.

For the sake of argument, however, assume that the above limitations can be removed: that is suppose that channelling in the crystalline electrodes keeps muons from the high- $Z$  atoms and the deuterium density is so high that d-d collision rates do not limit the process. The lifetime of a muon would then be sufficient to catalyse 1,000 fusions. But this also assumes it is continuously available for catalysis. In fact, in 8 per cent of d-d fusions the muon sticks to the  $^3\text{He}$  to form muonic helium<sup>12</sup>. Although in subsequent collisions of the muonic helium with deuterium, the muon could be stripped this will happen for only 32 per cent of captured muons, even with very high deuterium density<sup>13</sup>. (The high- $Z$  elements have large stripping cross sections, but stripping in this case often results in capture of the muon and so is not helpful<sup>14</sup>.) Hence, even under the most favourable circumstances, a single muon would catalyse only about 20 d-d fusions (or 340 d-t fusions). With the above muon stopping rate and assuming an electrode mass of 3 g, the neutron production rate would be  $2 \times 10^{-3} \text{ s}^{-1}$ , well short of the  $0.41 \text{ s}^{-1}$  reported by Jones *et al.*<sup>1</sup>.

Of course, if the fusion is catalysed by some stable charged quasiparticle in the electrode, one no longer has to contend with the short lifetime encountered with the muon. This can even offset the slower fusion rate that is inevitable with quasi-



### Fusion in 1926: plus ça change

ON September 17, the *Morning Post* published a Reuter message from Berlin to the effect that Profs. Paneth and Peters of that city had, after years of experimenting, succeeded in transforming hydrogen into helium "with the aid of particles of metal." This announcement, if correct, is of great importance and will evoke even more interest than the claim by Miethe and Stammreich to have transmuted mercury into gold.

No particulars are yet to hand concerning the methods adopted by Profs. Paneth and Peters, for the statement "with the aid of particles of metal" is meaningless as it stands. The experimental difficulties must be very great, not only in obtaining the energy necessary for such a change, but also in applying it under the appropriate conditions. Moreover, helium is an atmospheric gas, and traces of it are difficult to eliminate by the methods of evacuation and adsorption at present in use; belief or disbelief in the Reuter message must be reserved pending further and more definite evidence.

\* \* \*

PROFS. F. Paneth and K. Peters describe in outline how they have succeeded in detecting the presence of very minute amounts of helium, of the order of one hundred millionth of a cubic centimetre, derived from hydrogen which had been absorbed by finely divided palladium at the ordinary temperature.

Finely-divided palladium, either as sponge, 'block' or palladinised asbestos, was used to absorb hydrogen at room temperature. The residual gas obtained after a 12-hours' contact between palladium and hydrogen exhibited four or five lines of the helium spectrum; there was also a distinct proportionality between the amount of helium observed and

the duration of the time of contact. The activity of the different palladium preparations employed varied considerably; it invariably diminished with repeated use. No helium production was observed with palladium preparations that did not absorb hydrogen. They were not able to detect any trace of the energy liberated during the transformation, and they point out that the amount set free from the conversion of such small quantities of hydrogen — about 0.28 calorie — would be extremely difficult to detect, particularly if thermal changes due to absorption or formation of compounds also take place. They incline to the view that the liberated energy is more likely to appear as radiation,  $\gamma$  or Millikan-rays, than as heat.

\* \* \*

A FEW months ago, K. Peters and I published an account of experiments we had made in an attempt to transmute hydrogen into helium (*Ber. d. Deutsches Chem. Ges.*, 59, 2039, 1926). As a result of further experiments, we are in a position to give an explanation of the occurrence of the observed very small quantities of helium in our experiments without having recourse to the assumption of a synthesis of helium.

In the communication we discussed the possibility of regarding the helium dissolved in the glass as an explanation of the observed effects, but blank experiments led us to the conclusion that the quantity of helium liberated in this way was beyond the sensitivity of our method of detection. In the interval we have carried out experiments that show that the liberation of helium from glass is dependent on the presence of hydrogen. Thus glass tubes which gave off no detectable helium when heated in a vacuum or in oxygen yield helium in quantities of the order of  $10^{-6}$  c.c. when heated in an atmosphere of hydrogen.

As a result we have thus established that, in using an apparatus made of glass, one cannot make any trustworthy statement as to the origin of  $10^{-9}$  c.c. of helium if air comes in contact with the apparatus.

From *Nature* 118, 455 & 556 (1926); 119, 706 (1927).

particle masses less than that of the muon. Van Siclen and Jones's earlier calculations<sup>4</sup> suggest that reducing the hydrogen bond length by a factor of 2 (we believe a larger factor is needed), achievable with a quasiparticle with twice the electron mass, might give the neutron production rate reported now by Jones *et al.*<sup>1</sup>. Large effective electronic masses are often associated with transition metal crystals like palladium; unfortunately, the notion of heavy electrons is related to the non-local interactions of electrons with the crystal lattice; it is not easy to see how the local concept of tight binding can be squared with this.

The neutron rates observed by Fleischman and Pons<sup>2</sup> present further difficulties. Not only are they much higher, at about  $10^3 \text{ s}^{-1}$ , than those of Jones *et al.*, but they are a factor of about  $10^3$  less than expected from the rate of d-d fusions necessary to generate the heat they report. Thus they suggest some form of aneutronic fusion is occurring: for example a preference for

reaction 1a over 1b.

The equality of rates for 1a and 1b given by charge symmetry of nuclear forces is slightly altered by Coulomb 'isospin' mixing (G. M. Hale, personal communication). For bare nuclei (plasma fusion) the p + t product channel is very slightly favoured, but for muon-catalysed fusion the  $n + ^3\text{He}$  channel is favoured by a ratio of 1.4 (ref. 15). The latter is a consequence of fusion taking place in a state of unit angular momentum. In any event, it would seem quite difficult to suppress the neutron channel or to hide the tritium from assay. The normally rare radiative channel,  $d + d \rightarrow ^4\text{He} + \gamma$  (20 MeV), would also show up in the lower-energy  $\gamma$ -ray detector from pair production. On the other hand, only heat would be detected if it were somehow possible for the energy to go directly into the palladium crystal lattice ('Mössbauer fusion'), but such a process is expected to be strongly suppressed by the vastly different energy scales of the crystal lattice (eV) and fusion



## ECOLOGY

(MeV). Likewise, as indicated by the small branching fraction for reaction (3b), transfer of all the energy to a heavy electronic quasiparticle, even if it exists, is highly unlikely unless it is even more massive than the muon. Curiously, an excess amount of  $^4\text{He}$  did show up in the assay done by Fleischmann and Pons.

The above difficulties in interpreting the Fleischmann and Pons experiment in terms of d-d fusion lead one to consider the possibility of other nuclear reactions that might not produce neutrons or tritons. The electrolyte contained lithium so that  $^6\text{Li} + \text{d} \rightarrow ^4\text{He} + ^4\text{He}$  fusion is possible at the electrode surface or with long lithium drift times into the palladium; the intermediate state,  $^6\text{Be}$  has a rich set of resonant energy levels to enhance the reaction, but it is difficult to see how the autronic reactions  $^6\text{Li} + \text{d} \rightarrow ^7\text{Be} + \text{n}$  and  $^6\text{Li} + \text{d} \rightarrow \text{n} + ^4\text{He} + ^4\text{He}$  could be suppressed. Another difficulty is the smaller penetrability that comes from the greater nuclear charge, the higher reduced mass and the larger bond distance. For similar reasons, a cold fusion of d with Pd can be considered highly unlikely. Here again we note that there is the possibility of reaction with p as well as d, namely  $^7\text{Li} + \text{p} \rightarrow \alpha + \alpha$ .

In all this discussion, we have presumed that the current observations<sup>1,2</sup> truly belong to the cold fusion family. We might point out that they could be but a distant cousin. For example, experiments show that d-d fusion neutron emission can accompany the fracture of a LiD crystal<sup>10</sup>; the interpretation is that deuterons are accelerated to kiloelectron volt energies by the strong electric fields at the propagating crack. Such an effect might occur in the embrittled Pd or Ti crystal, in which case we would have 'microscopically hot' fusion under very unusual conditions. Neutrons would be emitted in bursts, not continuously. Other means of creating cracks suddenly would lead to similar effects. □

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## Honeyguides and humans

Robert M. May

ONE of my favourite children's books is an engagingly illustrated account of some of the mutualistic associations found in nature. One such illustration is of the honeyguide — appropriately named *Indicator indicator* — perched on a branch over a bee colony being torn apart by a badger-like ratel. The ratel has been led to the hive by the bird, and after the ratel has finished feasting on the honey, the honeyguide will feed on fragments of honeycomb left behind. The honeyguide needs the ratel because the bee colonies are typically situated in large trees, rock crevices or termite mounds in such a way as to be inaccessible to the unaided bird; the ratel apparently benefits from the honeyguide's deliberately leading it to the source of honey. Isack and Reyer<sup>1</sup> have



Ardea/Peter Steyn

Young honeyguide leaving the nest.

just completed a 3-year study in which they document the involvement of a third species in this association — humans.

Rock paintings in the Sahara, Zimbabwe and South Africa show that humans have collected honey in Africa for at least 20,000 years<sup>2</sup>. Many anecdotes (the earliest dating back to the seventeenth century<sup>3</sup>) suggest that humans have cut into the honeyguide–ratel dance, using the bird to help guide them to honey sources. Sceptics have viewed these anecdotes as romantic myths, and the issue may soon become moot because in many areas honey is increasingly obtained from bee-keeping or is being replaced by commercial sugar or other products; in these areas, the birds no longer guide.

Isack and Reyer's study<sup>1</sup> of the interactions between honeyguides and nomadic

Boran people in northern Kenya is therefore timely and interesting. Treating this system as if it were any other field study of a mutualistic association, the authors show that, in unfamiliar areas, honey-hunting human groups take on average 3.2 hours to find each bees' nest when guided by a bird, and 8.9 hours when not guided. This roughly threefold reduction in searching efficiency without a honeyguide is a conservative estimate, because the figures do not include the many days of unguided search on which no nest was found.

Isack and Reyer also document the benefit to the birds: 96 per cent of the nests they saw discovered (178 of 186) would not have been accessible to the birds until humans had opened them with tools. In addition, the Boran's use of smoky fire reduces the bird's risk of being stung. Because of the pronounced benefits to both parties, it is not surprising that humans and honeyguides have elaborated upon the previous ratel–honeyguide association by developing their own inter-specific communication system. Humans attract the bird with a penetrating whistle that can be heard more than 1 km away; Isack and Reyer found such whistles doubled the rate at which birds were encountered. On its part, the honeyguide attracts human attention by flying close or hopping around among perches, emitting a characteristic double-noted call.

Once humans and birds are engaged in a cooperative quest, Isack and Reyer show that the bird leads in consistently direct routes to colonies up to 1 km or more distant. Isack and Reyer find quantitative support for Boran honey-collector lore, demonstrating that three measures of bird behaviour decrease with diminishing distance to the bee colony: (1) the length of time the bird disappears after the first encounter; (2) the distance between the perches where the bird waits until the follower has caught up; and (3) the height of such perches. On arrival at the nest, the bird perches close to it and gives a characteristic 'indication' call (this call, like the initial one, is documented in sonograms). The bird also hops among close perches, often circling the nest in between perching. Isack and Reyer remark that this behaviour of effectively reducing the 'step length' as the goal of the search is approached is another example of a pattern (often called area-restricted search) that is ubiquitous in nature, being found, for instance, in flies looking for sugar particles<sup>3</sup>, parasitoids searching for hosts to oviposit on or in<sup>3</sup>, or schistosome miracidia homing in on snails as intermediate hosts<sup>4</sup>.

How do the honeyguides find the nest to which they guide their ratel or human associates? One answer is that the birds know the location of colonies in advance, another is that the birds literally 'wing it' until the sight or sound of accidentally encountered bees gives a clue. The rather straight flight-paths recorded by Isack and Reyer argue indirectly for the former explanation. More directly, Isack and Reyer watched from blinds and saw several unaccompanied honeyguides visiting nests, apparently on tours of discovery and inspection (when the bees were relatively quiet, as on cool mornings, birds would actually fly into the entrance of a nest and peer into it).

Isack and Reyer's study is, of course, engaging in itself. But, as the authors observe, it also addresses a larger issue. Boran 'ecologists' knew all their conclusions, even though they had not buttoned them down with sonograms and Mann-Whitney two-tailed tests. To an even greater degree, in the tropical rainforests

of Africa, Asia and South America, about whose flora and fauna conventional science knows so little, a vast store of ecological knowledge resides among the diminishing groups of native people, who draw upon such knowledge for food-gathering, medicine and other aspects of daily life. Ethnoecology, ethnobotany and other such disciplines are in their infancy. They hold the promise of helping us answer important questions more quickly than will otherwise be possible. Yet these possibilities are disappearing, as the cultures of native people are being destroyed at an even faster rate than the forests and other places where they live. □

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## OENOLOGY

# In vino veritas

Robert W. Cahn

GRAPE juice is often 'enriched' with additional sugar, in the hope of enhancing the quality of the wine resulting after fermentation. The European Community (EC), in the shape of the Council of Ministers, occasionally debates whether or not to prohibit the practice, or to limit the amount and type of sugar that may be added, and new regulations will eventually be promulgated. However, such regulations would be pointless without a reliable method to detect the added sugar, after fermentation is complete. Such a method now exists<sup>1</sup>, and the EC recently approved it as a standard Community procedure.

Jean Antoine de Chaptal (1756–1832)

was an agile French chemist who managed to keep his head while an eminent contemporary, Lavoisier, lost his to the post-revolutionary Terror. A landowner, Chaptal introduced the cultivation of sugar beet, the British blockade having prevented the normal importation of cane sugar. Having succeeded in this, he proposed that after summers with too little sun, grape juice be fortified with beet sugar so that it fermented as it should. Ever since, the process of fortifying wine in this way has been known among the French as *chaptalisation*. The *Supplement* to the *Oxford English Dictionary* defines chaptalization more broadly: "to convert or improve the must, in wine-making, by neutralizing an excess of acid or adding sugar". In the EC's Council of Agriculture Ministers, the French and the Germans, dwellers in cool climes, are inclined to refer to it informally as enrichment; the British, more neutral in the matter, call it sugaring; and the Italians and Greeks, whose hot climates ensure that grapes are never short of sugar, complain of adulteration.

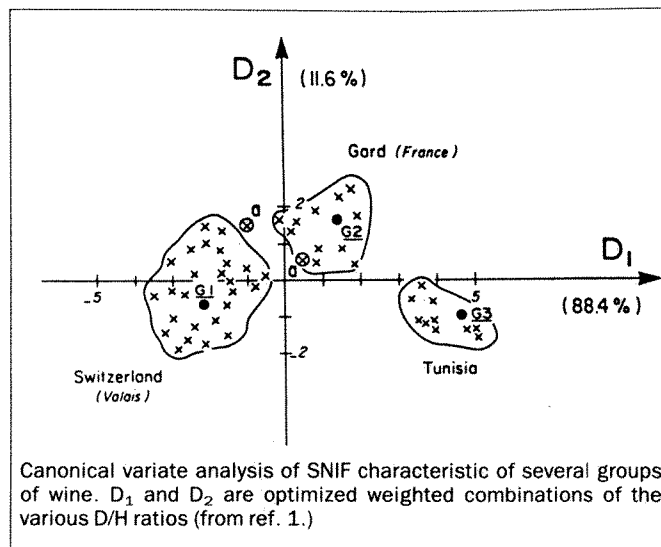
Whichever word is preferred, the

Commission has concluded that recent developments due to Gérard Martin's team at the University of Nantes will allow chaptalization to be identified *ex post facto*. Indeed, the French authorities, ever concerned to protect the reputation of French wines, some years ago offered a prize of 1 million francs for an effective method of detection, and Martin was the winner. Martin's most complete account of his technique was published last year<sup>1</sup>.

Characterization of wines and distilled liquors has for a long time used one of two approaches: either sensory analysis, which is an impressive name for tasting and sniffing (see illustration), with a top-dressing of multivariate statistical analysis and psychology, or capillary gas chromatography, which identifies the numerous esters and other constituents which are responsible for flavour. These approaches are critically discussed in a recent book<sup>2</sup> and clearly have serious weaknesses — not least, the difficulty arising from the fact that the population of esters, and so on, is constantly changing as a wine matures. In the same book, Williams<sup>3</sup> in particular examines the problems involved in tracking down a wine to its region of origin and in testing for chaptalization, and points to the special strength of the method introduced by Martin 'site-specific natural isotope fractionation' (SNIF).

The analysis of isotope ratios has, of course, become steadily more prevalent in geochemistry, environmental sciences and biochemistry; as practised in these disciplines, the normal tool is the mass spectrometer and isotope ratios refer to molecules or crystals as a whole. Attempts have been made to use the <sup>13</sup>C/<sup>12</sup>C ratio to distinguish between sugars from different sources (grapes, cane, beet), as different plants follow different photosynthetic cycles. It is possible to distinguish with some confidence between wines fortified with cane sugar and unfortified ones<sup>4</sup>, but it seems that beet sugar poses an insoluble problem with this technique (though the adulteration with beet sugar of rum, made from cane sugar, is detectable).

The drawback of the traditional method of isotope analysis is that only the overall isotope ratio in a molecule can be measured. Martin's method overcomes this limitation by measuring the proportion between hydrogen and deuterium in a specific site within an organic molecule by nuclear magnetic resonance. This type of measurement can be used to 'fingerprint', in particular, alcohols fermented from different sugar precursors. Ethyl alcohol has two distinct carbon-bonded hydrogen sites, the methyl and methylene sites, and by measuring D/H ratios for these sites separately, as well as for the water constituent of wines, Martin and co-workers can pinpoint the botanical and (to a somewhat lesser degree of reliability) the geographical origin of the alcohol. ▶





sentir le bouchon: olfactory sensory analysis will soon be displaced by SNIF-NMR<sup>1</sup>. (From *l'enseigneur le Vin*, Etablissements Nicolas, Paris, 1927.)

Martin and his wife, Maryvonne, first proposed<sup>4</sup> the SNIF-NMR method in 1981 and gave a much more detailed account<sup>5</sup> in 1986. In the meantime their son, Gilles, set up a company, Eurofins, in Nantes to exploit the patented technique, and the general assembly of the *Office International de la Vigne et du Vin*, a long-established intergovernmental body with 33 member states, in January 1987 after 4 years of tests approved SNIF-NMR as an officially recognized method of "displaying the chaptalization of wine" and the EC followed suit the following year.

In the SNIF-NMR method, the wine is distilled to obtain almost pure alcohol; this is necessary to enhance the precision of the NMR measurement, which depends on the alcohol concentration in the NMR sample. Recent research has established that, in general, results are more consistent if measurements are made only on the alcohol resulting from fermentation, not on the original must or grape-sugar solution. In fact, a recent (April 1988) resolution of the *Office International* has specified that only alcohol analyses be used for its purposes. The D/H ratios, which can generally be determined to a precision of 0.2 parts per million from the relative intensities of NMR lines, each identified with a distinct deuterated 'isotopomer', are calibrated against internationally standardized water samples<sup>6</sup>, against standard tetramethylurea and against a collection of sealed standardized alcohols fermented from different precursors, provided by the Community Bureau of References. Of course, the total weighted D/H ratio for an alcohol molecule taking into account all sites should match the D/H ratio obtained by mass spectrometry. According to Martin *et al.*<sup>1</sup>, there is indeed quite good agreement but with a systematic deviation of a few parts per million, which has recently been traced to differences in the deuterium ratio of the mutually exchanging hydroxyl sites of

water and ethanol (G. J. Martin, personal communication).

Over several years, Martin's team analysed many certified wines from France and other countries represented by the *Office International*, seeking correlations of the D/H ratios (for the methyl and methylene sites in alcohol and for water) with factors such as grape variety, region of origin (and hence climate) and of course chaptalization. The statistical method used is canonical variate analysis, aided by a specialized computer diagnosis system, ISOLOG<sup>7</sup>. Different weighted combinations of experimental variables (here, D/H ratios) are systematically compared to find the one best correlated with a particular external factor. The figure shows the results of the test of the distinguishability of groups of wines from the south of France, Switzerland and Tunisia, and the statistical separation is clear enough. The method can often distinguish between wines made from different grape varieties. The D/H ratios seem to vary according to the sugar concentration in the various grapes, which itself is a function of geography, grape variety

#### ELECTRON TRANSFER

## Energizing protons in membranes

R. J. P. Williams

In 1961, two proposals were made as to the way in which electron-transfer reactions of the cytochrome chain — the chain used in the oxidation of NADH by molecular dioxygen — could be connected to ATP formation without the intervention of chemical intermediates<sup>1,2</sup>. Both mechanisms invoked the transduction of the energy of the oxidation/reduction reaction to a proton gradient before the gradient generates ATP. The two mechanisms, sometimes termed the delocalized (Mitchell) hypothesis and the localized (Williams) hypothesis, are very different. In the first, protons generated by oxidation appear only in aqueous phases. Even ATP is generated by an electric field acting on the ATP synthetase and not by proton flow. In the second, protons move in proteins within matrices and aqueous phase equilibrations are ignored in the development of proton gradients, in proton diffusion and in the ATP-synthesis step. To distinguish between these mechanistic possibilities, long series of experiments have been carried out on separate parts of the cytochrome chain. The most decisive attack on the last stages of the electron-transfer reactions, those of cytochrome oxidase, has been carried out by Wikström and his collaborators<sup>3</sup>. Wikström reports his latest data on page 775 of this issue<sup>4</sup>.

Wikström and colleagues first showed that apart from the protons which react

and the weather preceding a vintage.

As things stand, Community regulations permit enrichment of grape must only with other (more concentrated) grape must, natural or rectified, except in those countries having a long tradition of adding beet sugar, which are free to follow their national rules. The Council of Ministers some time ago instructed the Commission to report to it on current enrichment practices; this report is due soon, and then the Council will have to try to reach agreement on future regulations for Europe as a whole. It undoubtedly is an extremely sensitive political issue. □

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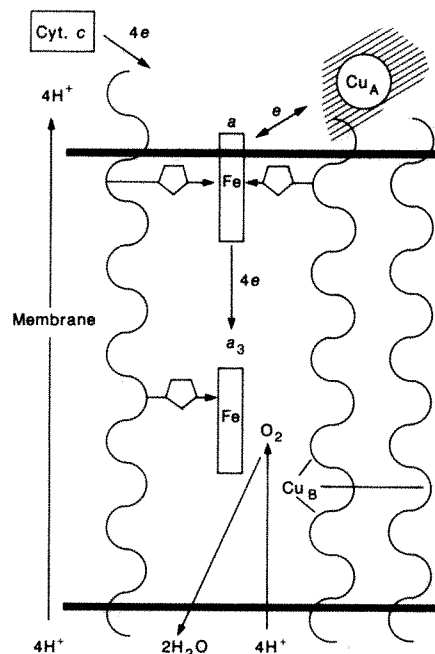


FIG. 1 Cytochrome oxidase (refs 5,6). The reaction centre binds dioxygen which is converted to water by the removal of protons from one side of the membrane (bottom). All the dioxygen reactions take place at the  $a_3$ - $\text{Cu}_B$  site while electrons come into the site from cytochrome *c* via haem *a* and  $\text{Cu}_A$ . The protein has several (putative) transmembrane helices and some, not all shown, are involved in proton pumping. One  $\text{H}^+$  for each electron is used stoichiometrically.



## Blow the fuse!

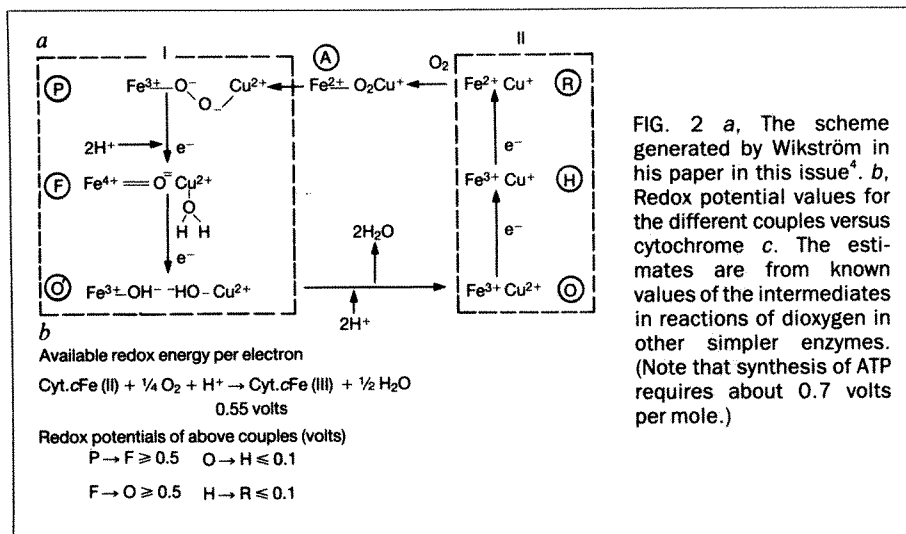


FIG. 2 *a*, The scheme generated by Wikström in his paper in this issue<sup>4</sup>. *b*, Redox potential values for the different couples versus cytochrome *c*. The estimates are from known values of the intermediates in reactions of dioxygen in other simpler enzymes. (Note that synthesis of ATP requires about 0.7 volts per mole.)

directly with dioxygen and which must diffuse through the protein matrix to the reaction centre in the membrane, additional (one H<sup>+</sup> per electron) protons are in fact energized by the reactions of molecular dioxygen in the membrane, and that these protons are pumped (in proton channels) across the membrane by gated energized diffusion<sup>3</sup>. The nature of the gating is as yet unknown. In an effort to elucidate the reaction path, Wikström now considers more precisely the coupling steps between the reduction of dioxygen by electrons and this proton pumping<sup>4</sup>. He concludes that proton pumping, which, note again, is separate from the protonation steps of dioxygen itself, occurs in association with the reaction steps involving only the first and second electrons of the four required to convert O<sub>2</sub> to 2H<sub>2</sub>O. I shall now consider some consequential mechanistic features.

In a protein such as cytochrome oxidase, consisting in all probability of membrane helices<sup>5,6</sup>, cooperativity should occur via conformational changes on O<sub>2</sub> uptake or electron transfer as in haemoglobin and haemocyanin. In fact the a<sub>3</sub>Cu<sub>B</sub> centre of the cytochrome oxidase can be thought of as half haemoglobin and half haemocyanin (Fig. 1). Now the reactions of these proteins and of oxidases such as cytochrome P<sub>450</sub> and even of cytochrome *c* with ligands are known to occur from one side of the proteins only via groove-opening reactions. It is hardly surprising, then, that the reactions of cytochrome oxidase, O<sub>2</sub> + 4e<sup>-</sup> + 4H<sup>+</sup> → 2H<sub>2</sub>O, occur through the oxidase from only one side of the membrane in which the protein is lodged. The apparent surprise is that the additional proton pumping in a different, helical channel is driven by the first two steps of reduction only.

Let us consider the steps. Each of the steps R → P, P → F, F → O, O → H and H → R are expected to be linked to conformational changes (Fig. 2 and see Fig. 1). The step R → P is not reversible. Whatever the series of conformational switches, the

later steps return the protein to its initial state in a cyclic, irreversible manner and so cannot be the forward strokes of a proton pump. Again, the redox energy of dioxygen relative to cytochrome *c* is largely lost going from P → O because the last two one-electron potentials of the oxidase are not far from those of cytochrome *c* itself (Fig. 2). It is good sense that the cyclic functioning of a pump has highly energy-using initial steps and later relaxation steps driven by little energy loss.

How does this pump compare with other ion pumps, such as ATP-synthetase, Na<sup>+</sup>/K<sup>+</sup> ATPases and Ca<sup>2+</sup> ATPases, and bacteriorhodopsin? In all these cases it has been supposed that cyclic helix/helix motions of proteins are part of the mechanical device in the pump. Moreover, it seems that the sites of energy input (here of ATP to the ion movements) are far in space from the gated channels, which are frequently considered to be constructed from anionic sites on helices within the membrane. The parallels with cytochrome oxidase are fairly close. Moreover in several cases where intermediates, such as phosphorylation of proteins, have been seen, the last steps, now loss of inorganic phosphate, act not in the pumping stroke but in relaxation, and release little energy.

It seems that several pumped movements of ions and possibly molecules in proteins in membranes have common features — helical protein structures, cycling conformation changes and readily distinguishable forward pumping and return steps where energy commitment is largely in the first steps. □

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LABORATORIES all round the world are trying to replicate the claimed electrolytic cold nuclear fusion from heavy water. Some may regard this as premature when the one crucial control experiment, the equivalent electrolysis of light water, has not yet been tried. But with his usual optimism, Daedalus has set DREADCO's electrochemists to forcing deuterium into palladium electrodes, and is determined to interpret any excess heat production as nuclear fusion, rather than some banal chemical or lattice effect.

Palladium has to take up a lot of deuterium before any heat is disengaged at all, but thereafter things seem to accelerate rapidly. This makes obvious sense: each site in the palladium lattice will take up a deuterium atom until all the sites are full. Further deuterium atoms will then enter already-occupied sites, and these close-packed pairs stand a chance of fusing together by quantum tunnelling. The energy released can, apparently, even melt the palladium.

But Daedalus goes further. He points out that as two nuclei approach each other, the chance of successful quantum tunnelling rises dramatically. So a sphere of deuterium-saturated palladium would make an ideal implosion-style hydrogen bomb. At atmospheric pressure it would release a steady heat flux and an inconvenient but containable neutron flux. Surround it by shaped charges of high explosive and crush it suddenly to half its volume, and the result should be a splendid combined fusion explosion and neutron emission. Despite the deadly momentary neutron flux, this elegant bomb would release almost no enduring fallout: a valuable military advantage. Lacking a critical mass, it could also be made in usefully small sizes.

Daedalus is rather diffident about contributing to weapons research. In self-defence he claims that nuclear physics is supported so lavishly by national governments only because it once produced a bomb. To keep the funds coming in, the subject really has to repeat the trick from time to time. In any case a pure, hygienic, noble-metal fusion weapon is a great ecological improvement on nasty poisonous and radioactive fission weapons, and should be supported enthusiastically by the increasingly militant 'green' political parties. But in more peaceful vein, Daedalus is also devising a fusion-powered watch. Each time its oscillating palladium hair-spring flexes, deuterium fusion accelerates on its compressed face, and slows down on its tensioned face. The differential thermal expansion unwinds the spring; the inertia of the balance wheel drives it the other way, generating the opposite thermal gradient, and so on. A tiny charge of deuterium should run it for centuries. David Jones

## Cold fusion: what's going on?

**SIR**—A significant point which is not widely known, and may therefore be overlooked in neutron measurements of cold fusion rates, is the possibility of contamination by cosmic-ray-generated neutrons; these should be taken into account in the design and interpretation of experiments.

The cosmic-ray-induced neutron background arises primarily from extra-solar protons with energies above a few GeV, which can penetrate the Earth's atmosphere and the Sun's and Earth's magnetic fields<sup>1</sup>. Primaries and secondaries reaching the surface include neutrons and other energetic particles which produce neutrons in the atmosphere and the first few metres of the surface by spallation reactions. While the spectrum contains neutrons up to energies comparable to incident particle energies, a major component is due to evaporation of neutrons from struck nuclei; at birth these have energies in the range 1–3 MeV, and appear as a knee or shoulder on an otherwise continuous energy distribution. There is also a substantial component consisting of 'thermal' neutrons, which have slowed down in the environment to a poorly equilibrated thermal distribution below energies of about 0.1 eV as well as 'epithermal' neutrons whose distribution is roughly inversely proportional to the energy in the range 1 eV to 1 MeV. At energies above a few MeV, the spectrum tails off rapidly; this cascade component contains about 10% of the total. The flux of each of the low-energy components is of the order of  $10^{-2}$  neutrons  $\text{cm}^{-2} \text{s}^{-1}$  at sea level and middle latitudes.

These figures vary according to altitude (about twice as great at 1,500 m elevation), and from time to time, mostly because of variations in atmospheric density and solar and geomagnetic field intensity. The e-folding thickness in the atmosphere is about  $150 \text{ g cm}^{-2}$ , so that, for example, barometric pressure variations of  $\pm 13 \text{ mm}$  of mercury cause about  $\pm 10\%$  flux variations. Sometimes, when dealing with such a general source of neutrons, material intended as shielding, and even detector material itself, can act as a source, so that some care in this respect is called for in the measurements.

As it happens, the counting rates due to cosmic-ray-induced neutrons are of the same order of magnitude as the counting rates observed in the neutron and secondary radiation detectors in many of the measurements being made. And in detectors that disperse the spectrum, the evaporation peak in the energy distribution due to cosmic-ray-induced neutrons is at nearly the same energy as that expected from deuteron–deuteron fusion, 2.45 MeV. These observations, coupled with the (admittedly weak,  $\pm 10\%$ )

temporal (hourly, daily) variation of the cosmic-ray-induced neutron fluxes require that this background be carefully accounted for.

Comparable neutron fluxes can be generated by accelerators, isotope sources and nuclear reactors, even at considerable distances; these contaminants of neutron measurements must also be reckoned with. Obvious means for suppressing these backgrounds are time-gating of the source, monitoring spurious sources with a second detector operated simultaneously with the detector(s) near the source under investigation, or going underground—350  $\text{g cm}^{-2}$  (two or three metres of earth or concrete on all sides) should reduce the cosmic-ray neutrons by a factor of about 10.

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**Dr Carpenter, a referee of the paper by Jones *et al.* on page 737, provided this comment at our invitation.**

**The following are extracts from the substantial numbers of letters from readers offering explanations of the two series of cold fusion experiments which have been generally reported.**

**Editor, *Nature*.**

**SIR**—From the newspaper accounts, the very small flux of neutrons generated during the experiment of Fleischmann and Pons is being taken as proof that their conclusion is not valid, and that nuclear reactions between deuterons do not occur under the conditions they describe.

But when the kinetic energy is as small as in their experiment, the neutron and proton components of the deuteron do not behave in the same way, because the nucleus of the target atom repels the proton but not the neutron. Thus, the neutron can be captured by the target nucleus while the proton, which remains outside the Coulomb barrier, will fly off.

This process, first recognized by Oppenheimer and Phillips<sup>1</sup> in 1935 leads to a pure (*d, p*) reaction and has a relatively high probability of occurrence, certainly much greater than that of the (*d, n*) reaction<sup>2</sup>.

It follows that if the experiments described really brought the deuterium nuclei close enough together to interact, one should expect no neutron emission and a reaction rate much higher than that evaluated on the basis of the high-energy

model.

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**SIR**—The only recognized mechanism for nuclear fusion at ambient temperature is that induced by negative muon binding of precursor hydrogen isotope molecules as first described by Frank<sup>1</sup> with estimates of fusion rates by Sakharov<sup>2</sup> and experimental observations by Alvarez<sup>3</sup> some 30 years ago. It is now known that more than 100 fusion events may be induced by a single muon during its lifetime of  $2.2 \times 10^{-6}$  seconds in a mixture of liquid deuterium and tritium<sup>4</sup>. If this number could be increased by a factor of 1,000, a break-even fusion reactor could be the result<sup>5</sup>.

It is tempting to interpret the recent claims in terms of this process. It should be remembered that more than 70 per cent of the cosmic-ray flux at the Earth's surface consists of positive and negative muons. There are about  $200 \text{ m}^{-2} \text{s}^{-1}$  with a stopping rate in an absorber of some  $2 \times 10^{-5} \text{ g}^{-1} \text{s}^{-1}$ . The flux may be twice as great at the altitude of Salt Lake City<sup>6</sup>, which would be equivalent to more than two muons a minute in an absorbing volume of about a litre.

The rate at which fusion events could occur is limited by the rate of formation of muon-bound molecules, which is itself a sensitive function of parameters on the atomic scale, whence its dependence on resonance effects<sup>7</sup> and temperature<sup>8</sup>. Once a muon is captured, the resulting muonic molecule is two orders of magnitude smaller than the typical lattice spacing in solids, so that free diffusion may be expected.

The fact remains that muon-induced fusion has not yet been reported in metallic compounds of hydrogen isotopes, and indeed has been considered unlikely because of the preferential capture of muons by the heavier metal nuclei. On the hypothesis that this loss mechanism is suppressed by a resonance or band structure effect in deuterium-loaded palladium, it is possible to estimate the turnover number required to explain the effects which have been reported.

Jones *et al.*<sup>9</sup> observe a neutron count-rate of  $4 \times 10^{-3} \text{ s}^{-1}$  with a neutron detection efficiency of about 1% in a volume of 160 ml. If the neutrons observed are products of the reaction, in a muon–deuterium molecule, of two deuterons to yield  $^3\text{He}$  and a 2.45-MeV neutron, one should also allow for the equally probable reaction yielding  $^3\text{H}$  and a proton, which would not have been detected by the neutron monitor. This implies five fusion events per second per litre. The required turnover number is then of the order of 100, comparable with that already known for the case of a mixture of liquid isotopes, but significantly greater than that in pure

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deuterium.

The consequence is that fusion induced by cosmic-ray muons cannot be excluded as an explanation of the reports of radiation effects in palladium loaded electrochemically with deuterium, although the estimates of the rate of muon-induced fusion is much less than that required by the thermal observations of Fleischmann and Pons<sup>10</sup>.

These developments emphasize the need for experimental data on the effects of negative muons in solids, especially metal deuterides and tritides, which are at present lacking in the open literature. Arrangements are in hand to investigate the reported palladium-deuterium effects with a muon source of greater flux than that of the natural cosmic ray radiation.

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SIR—In my theoretical investigations of the electronic structure of the  $H_2^+$  molecule (*Phys. Lett.* **123**, 170; 1987), I have found that the two nuclei and the electron can form a collapsing quasi-molecule — a compound system whose dimensions decrease with time to zero. (The extreme case is when the electron is at the centre-point between the two nuclei.) In general, in collapsing molecules like these, the repulsive Coulomb interaction of the nuclei and the gas-kinetic pressure of the electron are less than the attractive Coulomb forces between the two nuclei and the electron. The closest approach of the two nuclei depends on the initial state of the electron, its binding energy and the mean value of the kinetic energy in particular.

The probability of the tunnelling effect is therefore identical with the probability of formation of a collapsing quasi-molecule. Thus it is clear that the electrons present in the matter are responsible for the Coulomb-barrier tunnelling, and that the process which has been observed depends on quasi-molecular systems.

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SIR—Reports of the experiments by Fleischmann and Pons contain a paradox

— that, if fusion reactions do occur in them, either too much energy is liberated or too few neutrons are detected. I wish to suggest a possible explanation.

I start from the hypothesis that the palladium contains regions where the density of deuterons is sufficiently great for d-d fusion to occur by one of the reactions leading either to  $^3H$  and a proton or to  $^3He$  and a neutron. The product particles will be produced within a region where the density of other particles is very great. The mean free paths of the particles will then be very small, and it appears reasonable to assume that even though these high-density regions will be geometrically small, they will be so optically large that even the most penetrating particles, such as neutrons, will remain trapped inside them. In this situation, the particles produced by fusion reactions will undergo multiple scattering collisions until a new reaction occurs.

Several such reactions are possible, including fusion reactions of deuterons with  $^3H$  and  $^3He$  (yielding  $^4He$  and a neutron or proton respectively) and the radiative capture of protons or neutrons by deuterons. These reactions are exothermic, releasing large amounts of energy.

It is crucial that these processes can also form multiplicative chains, especially if the  $\gamma$ -ray photons released by radiative capture reactions yield further energetic neutrons and protons by the photodisintegration of deuterons. The reaction chains will come to an end only when reactive particles escape from high-density regions to those where the density is insufficient to sustain them.

The main products of these reaction chains will be  $\alpha$ -particles, but the reactive particles such as neutrons and  $^3H$  will only infrequently be released to the environment.

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## Pulsar formation

SIR—Lindley<sup>1</sup> states that the report<sup>2,3</sup> of a half-millisecond pulsar in the remnant of supernova 1987A "has surprised everybody and could, if confirmed, stand theory on its head". He has apparently overlooked several papers (refs 4 and 5, for example) suggesting that pulsars may form directly as rapidly rotating neutron stars "in original spin" with weak magnetic fields, without the need for "resurrection" after birth as a slow rotator followed by subsequent decay of the magnetic field and spin-up by accretion of matter from a companion star<sup>4</sup>.

Even before the discovery of millisecond pulsars, we discussed<sup>5</sup> the question of why collapsed stars rotate so slowly,

and pointed out that if there was significant mass ejection during the formation of a neutron star, and if it had a strong magnetic field, it was likely to be born spinning slowly. We argued, furthermore, that if the progenitor giant core of the neutron star has a strong magnetic field, it is likely to have been rotating relatively slowly, as the magnetic field would have enhanced transfer of angular momentum from it to the envelope during earlier evolutionary phases. We concluded that if weak-field neutron stars could form at all, they would be born spinning fast. If the optical emission from the half-millisecond pulsar arises from incoherent synchrotron radiation at the light cylinder, and scaling by the optical luminosity and magnetic field of the Crab pulsar, it follows that the half-millisecond pulsar indeed has a weak magnetic field of  $B \sim 10^8$  gauss.

With the discovery of six millisecond pulsars (with periods  $\leq 12$  ms), of which four are in binary star systems, several authors concluded that 'resurrection' was required to account for their observed properties. A key requirement for the 'resurrection' model is that the magnetic field of neutron stars must decay on a timescale of a few million years. But arguments against significant neutron-star magnetic-field decay have been proposed (for example, ref. 8). Furthermore, calculations by Sang and Chanmugam<sup>9</sup> showed that there are serious difficulties with all models so far proposed for field decay. In addition, there is no satisfactory detailed model that explains how single millisecond pulsars can be formed from binary star systems. Even in the case of PSR1957 + 20, where there is evidence of matter evaporating from the companion, the spin-down rate indicates insufficient energy loss from the pulsar to evaporate the entire companion star. When combined with all the difficulties of the 'resurrection' model, the discovery of the half-millisecond pulsar seems to provide substantial further support for the view that at least some millisecond pulsars can be born "in original spin".

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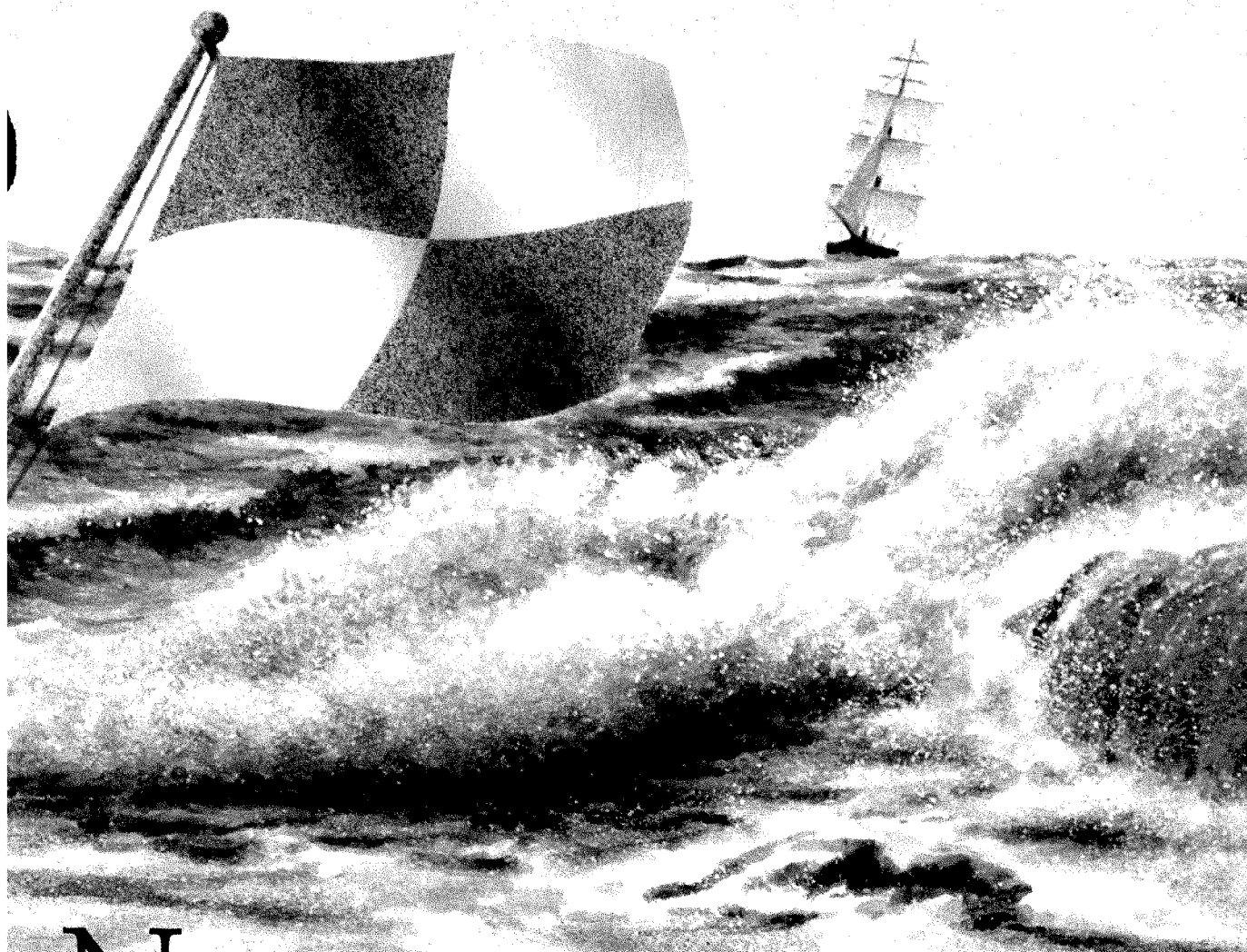
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# On the bright side

Steven Rose

**Biology and Freedom: An Essay on the Implications of Human Ethology.** By S.A. Barnett. Cambridge University Press: 1989. Pp. 376. £32.50, \$39.50.

I'M LEERY about books with the words 'biology and . . .' in the title — they tend to contain yet more angels-on-a-pin discussions by philosophers about the ethical implications of science-fiction genetics, or sociologically naive speculations by biologists about the inevitability of war, the nuclear family and capitalism. *Biology and Freedom* comes as a refreshing contrast. Professor Barnett is a distinguished ethologist of the school which pre-dates the present-day obsessions with optimal foraging strategies, cost-benefit analyses and genetic fitness calculations, and his previous books include an authoritative study on the social behaviour of the rat. As anyone who has had the good fortune to hear one of his forceful contributions to that great Australian radio institution, *The Science Show*, will know, he does not suffer fools lightly. But his new book goes beyond the mere debunking of certain currently popular biologicistic follies, to offer instead a vision of a humanity set free by our very biological constitution.

Barnett's intentions are perfectly revealed in the epigram from Shaw with which the book opens: "Is human nature incurably depraved? If it is, reading this book will be a waste of time". The first two-thirds of the subsequent text is devoted to a critique of those claims for such incurable depravity that have found their way into three decades of biological textbooks and Sunday-newspaper popularizations. The final third offers us the author's alternative vision. The targets for his criticism are designated respectively *Homo pugnax* (the claim that human beings are innately violent and aggressive); *Homo egoisticus* (the claim that we are innately selfish); and *Homo operans* (the claim that we are innately greedy).

Behind the first title shelter the pre-sociobiological generation of pop-ethologists such as Lorenz and Ardrey (in contradistinction to the recent obituaries, Barnett has no hesitation in referring to Lorenz's youthful Nazi enthusiasms and more recent New Right leanings). The claim of selfishness, of course, refers to the DNA-centric view of the universe offered by sociobiology's 'modern synthesis', and provides Barnett with the context for a synoptic account of neo-darwinian evolutionary theory. Greediness turns out to mask the skinnerian vision of human beings as driven exclusively by contingencies of reinforcement, of reward and punishment, as epitomized in the utopian/dystopian

vision of *Walden Two*. There have been numerous critiques of these positions, of course. E.O. Wilson himself described the writings of Ardrey and Lorenz as "mere advocacy". The sociobiologists have been criticized on scientific, ideological and philosophical grounds (this last especially by Philip Kitcher in his book *Vaulting Ambition*, which oddly does not appear in Barnett's extensive bibliography). Chomsky's polemic with Skinner is well known.

Barnett, bringing to these debates a combination of deep familiarity with the ethological and anthropological literature and a gentle wit, has little difficulty in exposing the shallowness of each of the three visions of humanity. He leaves unaddressed, however, the question of just why these views have achieved the popular resonance, and their authors the fame, that they undoubtedly have. Just why should such poorly predicated and evidentially weak ideas continue to be taken seriously? For some, the answer lies in the part they play in helping set political agendas; Barnett allows his readers to pose and answer such questions themselves. This reticence may be appropriate in a book which is evidently designed, as this one is, to serve as a teaching text in courses in the human sciences as well as for a wider audience.

It is to the last third of the book that one must turn to move beyond critique and to

arrive at the author's own vision of human nature, the vision which informs the book's title. Reductionism and determinism fail as methods of understanding the human condition because in considerable measure our future is undetermined — we make our own history, though in circumstances not of our own choosing. Human beings are playful animals, curious animals, animals who make music, animals for whom work is not necessarily labour but can be liberating, unalienating. And human beings are also animals who teach. The reciprocal pleasures of teaching and learning, of the relationships between adults and children, are beautifully conveyed, both in prose and in many of the photographs which enliven the book.

Barnett's message is unfashionably optimistic. Certainly science and technology are associated with great horrors — and with the peculiarly distorted visions which the first portion of the book is concerned to dispose of. Equally certainly, human perfectibility is not an option. When television screens are full of images of war and starvation, when fanatical fundamentalists can condemn to death a writer who dares to laugh, it is hard to hold on to a utopian perspective. But Barnett remains unshakably a progressive. We do our present age an injustice, he argues, if we cling to ideas of human beings as greedy, violent and selfish, and fail to realize not only how far we have to go, but also how far we have already travelled towards a free, egalitarian and democratic society, a society which it is in accord with our biological natures to create. □

Steven Rose is Director of the Brain and Behaviour Research Unit, The Open University, Milton Keynes MK7 6AA, UK.



Threatening gesture — the male three-spined stickleback *Gasterosteus aculeatus* in a posture that intimidates other males of the same species, here seen intimidating its own reflection. The behaviour is innate, and depends on both internal motivation and external sensory factors. The picture is taken from a reprint of *The Study of Instinct* by Nikolaas Tinbergen, who died last year (see S.A. Barnett's obituary in *Nature* **337**, 509; 1989). The book contains the original text written in 1949, along with an introduction added in 1969 and a brief 1988 preface. Publisher is Clarendon, price is £12.95.



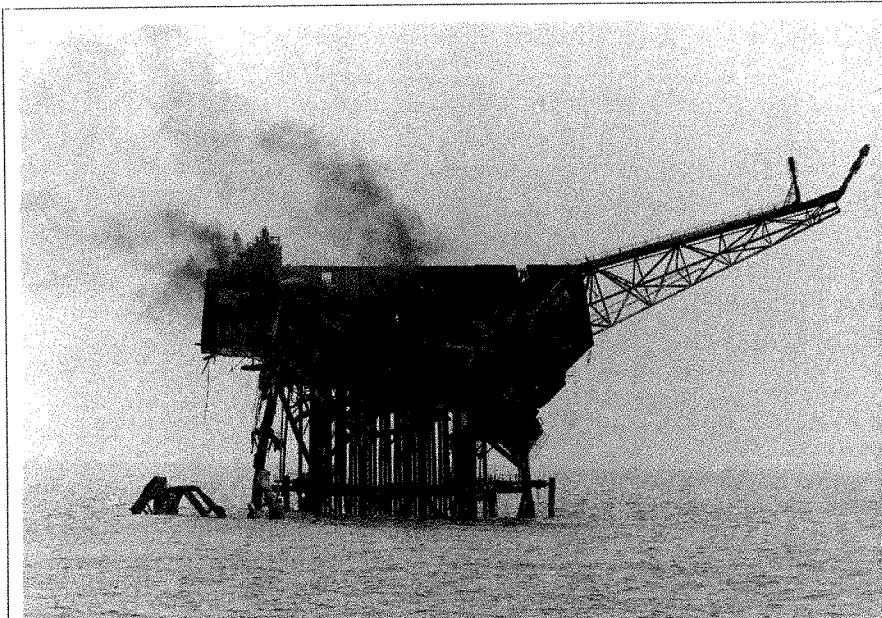
## Dirty deeds

John H. Simpson

**Pollution of the North Sea: An Assessment.** Edited by W. Salomons, B.L. Bayne, E.K. Duursma and U. Förstner. Springer-Verlag: 1988. Pp. 687. DM 198, £65.

LAST summer the seals of the North Sea died in their thousands as an epidemic of canine distemper swept through a population whose numbers had previously been increasing. The episode, accompanied by intense media attention (it was the silly season), served to alert us all to pollution in the North Sea.

Ironically, it now seems unlikely that there was any direct connection between the seal deaths and toxic pollutants. But the plight of such appealing creatures (jelly fish would not have been the same) served as a powerful focus for public fear that something is seriously wrong in the ecosystem of Europe's shelf seas, and that remedial action is needed to avoid catastrophe. Marine scientists are now rightly being pressed to state what exactly are the pressures on the North Sea and to what extent they constitute an urgent threat. In *Pollution of the North Sea*, a wide-ranging and timely survey, the editors have attempted to assemble the best available



Disastrous legacy — the explosion on Piper Alpha in July 1988 killed over 150 workers and may have released toxic compounds into the North Sea.

scientific answers to these questions, and to express what they identify as the remarkable consensus amongst the scientists involved as to the vulnerability of the North Sea and its finite capacity to assimilate waste. That does not mean that there is no disagreement about the details; with so little knowledge in some areas there is still plenty of room for different interpretations and hypotheses about mechanisms. But there is a determination manifest in this volume to establish a scientific assessment of the problem undistorted by national political pressures.

With 40 separate papers by more than 70 authors, the book covers most aspects of the subject. The first section reviews the basic physics, chemistry and biology of the North Sea, giving due weight to the importance of suspended particulates — these absorb many of the pollutants, carrying them into the sediment where they are exposed to diagenetic processes before possible resuspension brings them back into the water column. Also here is discussion of the role of the surrounding sediment traps (salt marshes, estuaries and fjords) which act as sinks for pollutants, and of the natural variability of the system which so much complicates the problem of detecting the incipient consequences of pollution.

Section 2 provides a useful compendium of facts and figures on the main inputs of pollutants into the North Sea including, for example, best estimates of how much cadmium, mercury and lead (the latter mainly from the atmosphere) are entering each year. Some of the inputs, notably of organic pollutants, are hard to quantify. It is difficult to determine the concentrations of such a large number of compounds, and there are uncertainties surrounding single catas-

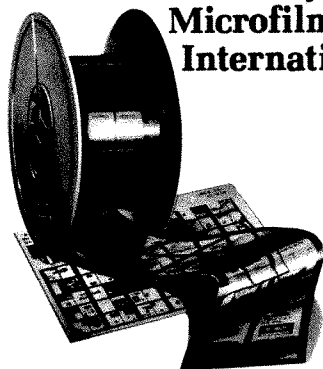
trophic events such as the Piper Alpha disaster, which may have resulted in the release of several tons of polychlorinated biphenyls, and the recent loss of a large quantity of Lindane in the Channel. The inputs of nutrients, on the other hand, are certainly increasing dramatically; the amounts of nitrate and phosphate coming from the Rhine double about every 15 years, and there is evidence of consequent eutrophication in the German Bight.

Pollutant effects in particular areas and on particular sections of the biota are considered in more detail in the two final sections. We learn, for example, about the specific problems in marginal areas such as the Wadden Sea, where pollution pressures are most intense and where we might look for early warning of the consequences. The Wadden Sea offers some of the few examples (for instance decline of the harbour seal, and changes in the populations of spoonbill and sandwich tern) where we have good evidence of the impact of pollutants on particular species.

Given so many contributors, the scientific quality of this large volume is inevitably somewhat variable. Although the choice of material is, on the whole, balanced, there has been no attempt at synthesis in the form of overall and sectional synopses. This, I fear, will make the book harder to use and less accessible to the non-specialist than it might have been. Nonetheless, *Pollution of the North Sea* is a unique and valuable compilation of material. It will serve as a vital source of reference for those involved in the science and management of the North Sea, and in the presentation of the attendant issues to a wider public. □

John H. Simpson is a Professor in the School of Ocean Sciences, University of Wales, Bangor, Gwynedd LL57 2DG, UK.

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# The opposite of knowledge

John S. Rigden

**The Privilege of Being a Physicist.** By Victor F. Weisskopf. W.H. Freeman: 1989. Pp.235. \$17.95, £12.95.

IT is appropriate that the most prominent word in the title of Victor Weisskopf's book is *Privilege*. Through talent and hard work, Weisskopf has gained an international reputation and has attracted many honours; yet he is that rare kind of man who regards it all as a privilege.

For readers familiar with two of Weisskopf's earlier books, *Knowledge and Wonder* (Doubleday, 1962) and *Physics in the Twentieth Century* (MIT Press, 1972), the appearance of this new work will be a happy event. It brings together 16 essays: two were written specifically for the book, the others have mostly appeared over the past ten years (although four of them have been expanded for the present collection). The subjects covered are some of the principal ideas of twentieth-century physics, two of the physicists who participated in the creation of those ideas, and the relationship of science to culture and to society.

The theory of quantum mechanics was created during the years 1925–1927. For those who followed events, it was a time of intellectual exhilaration and purgation. Conundrums that had plagued physicists during the early years of the twentieth century were suddenly resolved — provided they were willing to accept nature on entirely new terms and think in entirely new ways. To engage one's mind in thinking about such a conceptually rich subject, and to do so in the fundamental ways that were required to create the formalism of quantum mechanics, is an experience that alerts the mind to the broad place of physics in human activities.

With just a touch of sadness, Weisskopf has acknowledged that he came to physics "three years too late" — too late, that is, to be an active participant in the creation of quantum mechanics (*Physics in the Twentieth Century*, p.4). Starting in 1929, however, he made many seminal contributions through the application of quantum mechanics to our understanding of matter. Moreover, he has been one of those theoretical physicists who has thought deeply about the ideas of quantum mechanics and about the interpretation of this incredible theory. In addition, he knew personally men such as Bohr, Heisenberg and Pauli who were the architects of quantum mechanics and he bears their influences. This is the background that Weisskopf brings to his writing.

Although they were written for the

general reader, scientists will also find the essays edifying. Weisskopf does not present physics as an activity isolated from values and emotions. Quite the contrary. He shows us how physics and the humanities arise from the same universal values and complement each other. Teachers at all levels would be well advised to read and ponder the contents of the book. Too often science instruction takes the form of transmitting bits of knowledge that are isolated from any scientific, cultural or human context. But such bits of knowledge provide students with no under-



From *The Privilege of Being a Physicist*

Weisskopf — a rare kind of man.

standing of science. Weisskopf goes further: in the chapter "Teaching Science", he asserts that "science is the opposite of knowledge" and portrays science as the product of curiosity and questioning which can lead to a "fuller, more meaningful life".

Weisskopf is at his best when he writes about the ideas of physics. In "What is Quantum Mechanics?", written specifically for this collection, we learn how "the primal shapes of nature" bring stability to atoms and how the uncertainty inherent in nature really brings the certainty that renders all atoms of a given element identical. This essay, along with "What is an Elementary Particle?", "Contemporary Frontiers in Physics" and "The Origin of the Universe", reveals not only the depth of Weisskopf's understanding, but also demonstrates his expository skill. For

their clarity and insight, these essays are outstanding.

Wolfgang Pauli and Werner Heisenberg are each the subject of Weisskopf's attention. Pauli is typically the subject of many anecdotes; Weisskopf adds to them and, in the process, brings insights to Pauli's intriguing personality. In the case of Heisenberg, Weisskopf's admiration of the man prompts an explanation of his wartime activities; this account is based largely on *Inner Exile* (Birkhäuser, 1984), the recent book by Heisenberg's widow, and in comparative terms it is somewhat more generous than the findings of contemporary historians such as Alan D. Beyerchen (*Scientists Under Hitler*; Yale University Press, 1977).

Implicitly, Weisskopf extends the same generosity to American physicists. If quantum physics can be said to have had a determining influence in Weisskopf's life, so can his experiences at Los Alamos. In 1943, Hans Bethe persuaded Weisskopf to join the team at Los Alamos and work on the development of the atomic bomb. Weisskopf became one of the prime movers in the Manhattan Project. When the war ended, however, he refused to participate in the further development of nuclear weapons; rather, he helped to form the Federation of Atomic Scientists, a group whose purpose was to alert the public to the dangers of nuclear war. In making this decision, Weisskopf differed with many of his fellow physicists who on the one hand called for an end to the arms race and on the other continued to develop successive generations of nuclear weapons. As the history of the post-war period is now being written, it is apparent that one of the primary driving forces of the arms race has been the ability and willingness of physicists to realize technical possibilities which have inevitably proved irresistible to political and military leaders. In this regard, Weisskopf might have expanded his concept of the privilege of being a physicist to include more directly and more insistently the idea of responsibility. As for himself, Weisskopf's responsibility has been fulfilled: in the book he continues to call for an end to the madness of nuclear weapons.

The essays were written over a period of years, so there is some repetition; I suggest that each of them should be read individually (rather than consecutively). In this fashion, they will reinforce each other while each is savoured. □

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• Part 2 of Solly Zuckerman's memoirs, *Monkeys, Men, and Missiles: An Autobiography 1946–1988*, has been published in the United States by W. W. Norton. Price is \$22.50. The British edition was reviewed by William Cooper in *Nature* 336, 285 (1988).

## Pleasing all of the people

C. J. Gilmore

**General Chemistry.** By P. W. Atkins. W. H. Freeman: 1989. Pp. 989. Hbk \$49.95, £32.95; pbk \$39.95, £16.95.

WRITERS of textbooks on general chemistry have a problem that their colleagues in subjects such as biochemistry, cell biology and computer science (to name but three examples) do not have. Chemistry is an old subject, many of its foundations being laid in the nineteenth and early twentieth centuries. Thermodynamics, for example, is a product of the second half of the nineteenth century, though it remains as relevant now as it was 100 years ago. Quantum mechanics, as studied by undergraduate chemists, is over 50 years old, but is likewise a vital ingredient of modern chemistry.

At the same time the subject itself has not stood still. So how does one teach both the old and the new, and how is the old kept fresh and stimulating? And then there is the problem of conflicting requirements. Scottish, English and North American universities all have different entry standards, and give courses in the first year that demand different prerequisites, so the author of a general text has to decide the potential market that is being addressed — it is impossible to please all of the people all of the time. The market for such broad-brush textbooks is enormous; most publishers have one or more appropriate titles in their lists, yet most of them are virtually indistinguishable in their approach and presentation.

Peter Atkins is well known as an author of physical chemistry textbooks. His *Physical Chemistry* (Oxford University Press, 1986) is now in its third edition and is widely used by relatively advanced undergraduates. Another of his books, *Molecules* (W.H. Freeman, 1987), presents the concepts of molecular shape, so important in biological aspects of chemistry, in a novel and entertaining way. Atkins also has strong views about the topics which he feels should be taught; in an article published in 1987 (*Chemistry in Britain* 23, 640–641), he argued the case for teaching through computer modelling and provoked considerable debate. We have, therefore, an experienced author with some controversial views — in turning to general chemistry, has he produced something different from the standard texts?

The initial impressions are encouraging. The book's presentation is attractive, with one-third of each page reserved for a column which displays diagrams, photographs and other figures. Colour is used

well, although the pale-blue background to photographs of apparatus sometimes obscures the colour changes that are being shown. The pictures of molecules are especially good, with space-filling models used as in *Molecules*. This new book must rank as one of the best-produced chemistry texts I have ever seen.

The content, however, is pretty conventional with none of the innovation that one might have hoped for. By British standards, organic chemistry is handled only superficially: there is a lightning tour of the subject scampering through everything from alkenes to nucleic acids in two chapters of 70 pages. There is no attempt to introduce mechanism — yet how can students ever make sense of organic chemistry without it? For inorganic chemistry there is a similar tour of the periodic table, and these two factors alone make it clear that a North American audience is what the author and publisher have in mind. As

one might expect, physical chemistry fares best; over two-thirds of the text is devoted to the subject, with a commensurate increase in the academic standard.

There are some good touches — a description of how to quote the correct number of significant figures in numerical calculations, and a massive number of problems and worked examples. Indeed, the book is (or will be) accompanied by a study guide, an instructor's manual, a solutions manual (only the odd numbered numerical exercises have answers in the book itself), a laboratory manual, a computer test bank, a video demonstration and overhead transparencies. My prognostication is that, in a very full market place, *General Chemistry* is likely to be a best-seller in North America but less used in Britain.

C.J. Gilmore is a Senior Lecturer in the Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK.

## A closer look

Tom Mulvey

**Principles of Electron Optics.** By P.W. Hawkes and E. Kasper. Academic: 1989. Two volumes, pp. 1,188 plus indexes. Vol. 1 £37.50, \$84.50; vol. 2 £37.50, \$79.50; the two together £65, \$140. A third volume is in preparation.

IT IS 56 years since Ernst Ruska built the electron microscope that broke through the resolution barrier set by the optical microscope and later earned him the 1986 Nobel prize in physics. These instruments now have a huge influence on science and technology, diagnostic medicine, crime detection and food technology, and hence on society itself. The design of modern electron optical microscopes and the interpretation of the resulting atomic images is a scientific challenge. Electron microscopy makes great demands on theoretical physicists because it embraces the whole range of classical and wave optics, newtonian and relativistic mechanics, and quantum physics. Many former theoretical speculations such as 'quantum wells' can now be observed routinely.

A lot of books on the subject have appeared in the past, but *Principles of Electron Optics* is the first attempt, since the advent of the digital computer, to cover the entire subject systematically and critically. There are three volumes, each of about 600 pages. The first two, *Basic Geometrical Optics* and *Applied Geometrical Optics*, are reviewed here; Vol. 3, which will be concerned with wave optics, image interpretation and electron holography, is in preparation. The authors assume that the reader has a knowledge of physics and mathematics to

degree level, and aim to provide a self-contained, modern account of their subject for anyone 'involved with electron beams of modest current density in the energy range up to a few megaelectronvolts'.

In Vol. 1, the emphasis is on basic principles rather than instrumental applications. This, however, is no mere repetition of material to be found in textbooks — Hawkes and Kasper have reworked their material into a coherent and unified whole, making a careful appraisal of the best way to approach a given topic. Although the role of traditional analytical methods is clearly brought out, the impact of computer methods is rightly stressed. The authors compensate for some inevitable omissions by providing a comprehensive set of notes and further references directing the reader to the original literature. Volume 2 deals with the application of the theory to the calculation of the optical properties of lenses and other electron optical elements. Particularly valuable is the chapter on electron guns, a difficult and widely misunderstood subject.

This is a monumental and timely work — well researched, carefully proof-read, and marked by clarity of thought and expression. That it is in English and uses SI units is an enormous advantage for today's worldwide readership in electron optics and microscopy. It merits close study by all physicists and engineers concerned with the subject, and indeed by all those who wish to gain an insight into the power of theoretical physics to deal, often in a deceptively simple way, with the complex situations that now arise routinely in electron microscopy. □

Tom Mulvey is Emeritus Professor of Electron Physics at Aston University, Birmingham B4 7ET, UK.



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LN	Lawson Number	1
PRE	Preparation	2
MP	Melting Point	12
INP	Isolation from Natural Product	2
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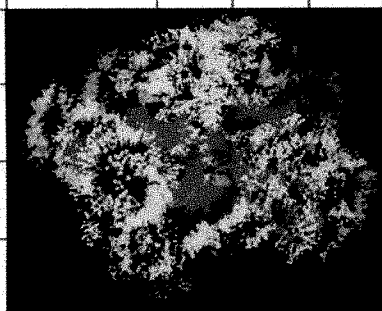
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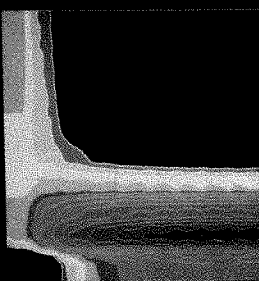
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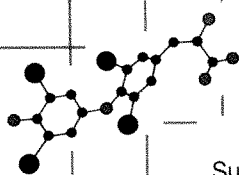
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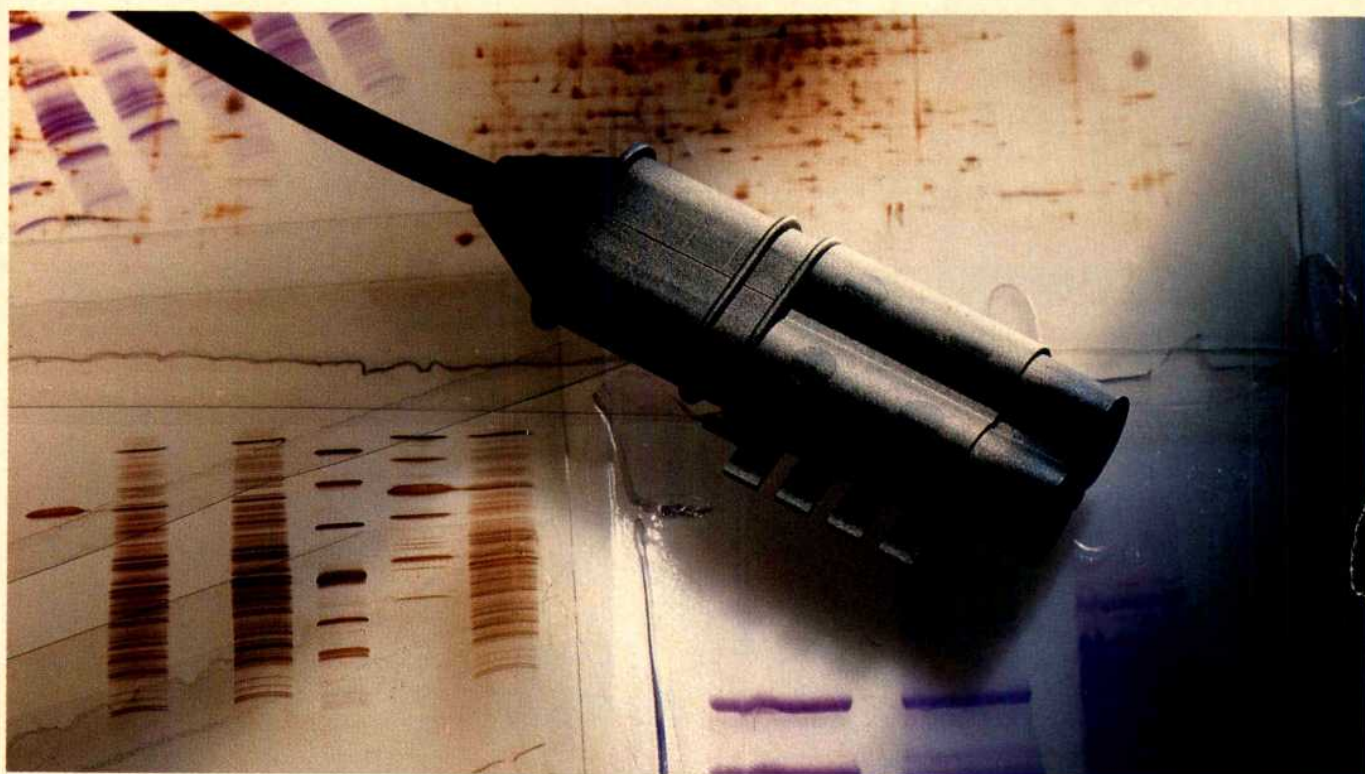


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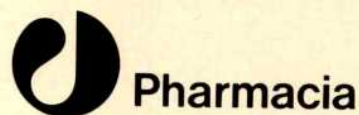
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# Collaboration in a wider Europe

Europe is in a mood to collaborate in research, as in much else, but doubts persist about the proper framework. This survey of science in Europe may resolve some issues.

EVERYBODY agrees that Europe is in transition, but the end-point is undefined. That is the starting point for the survey of science in Europe that occupies the following pages. Several important influences, cultural, economic and political, have conspired to provide an impetus for change that, in the long run, will help determine what the world is like, but the most interesting of them is the growing inclination of those who live in Europe to regard themselves as members of a community whose common interests transcend the chauvinistic differences that have bitterly divided them in the past.

That is why this survey differs markedly from others in this biannual eight-year series. It is not so much a record of achievement and disappointment in research, as of the mechanisms by which Europeans collaborate. And because there is so much going on, this will seem even more neglectful than previous surveys of important institutions contributing to the increasing cohesion of European science. It is hoped that those who are concerned with these institutions will not be too deeply offended.

Many of the institutions that now exist to foster European collaboration have their roots in the period immediately after the Second World War, and in the then common revulsion against the folly that had brought most European states economically to their knees. But there is an important sense in which the lesson also derives from the earlier 1914–18 war. In the settlement of that disaster, states called great powers redrew the map of Europe artificially while demanding that Germany should pay for the cost of the trouble it had caused — in retrospect a device for creating the conditions that made the Third Reich possible. That seemed a bad precedent in 1945. But by then, the great powers of the time had already (at Yalta) redrawn the European map so as to divide East from West. All the more necessary that the other ingredients of the settlement after 1918 should be avoided.

That is why the past 40 years have seen so many attempts in Western Europe to create a framework in which national interests and cultural differences would be subsumed within a common purpose. In rapid succession, there were formed the Western European Union (concerned with mutual defence), the Council of Europe (to foster common social and legal standards) and the succession of economic communities, which were at first concerned with commodities such as iron, steel and nuclear energy, but now, as with the twelve-member EEC, are concerned with the whole of economic activity. (The second half of this survey, beginning on page 726, deals with EEC science and what should be done about it.)

Collaboration is evidently infectious. The dramatic proof of that is Mr Mikhail Gorbachev's recent talk of the

"common European home". Gorbachev, who is not naïve, no doubt has many objectives. But suddenly, it seems, everyone wishes to collaborate. Poland, Hungary and Yugoslavia are talking of full membership of the Council of Europe, its Convention on Human Rights apparently not an obstacle. Turkey has applied for membership of the EEC, Austria is wondering whether to do so, while the Scandinavian countries and Switzerland may yet have a close relationship with the EEC.

Where will it end? And will the outcome revitalize European science? The continent (including its offshore islands) has never been full of optimists, which is why there is also now as great a need of realism. It is easier to decree collaboration than to ensure that it succeeds, especially in the practice of scholarship and research — and when the decrees are made by politicians. Here are some challenges and rules for optimists:

■ **Higher education.** In the 1860s, the great Ernst Mach left Vienna to teach at Prague, which is not very far away, but that would not now seem a natural move. Europe now has too many separate university systems. Even within the EEC, too many universities are too tightly in the pockets of their governments, national or even regional, yet there are no plans for integrating them. So how, and when, to make progress towards a wider framework?

■ **Basic research.** There are two frameworks of collaboration — within the EEC, and more extensive (typified by CERN and the European Science Foundation). Within the EEC, the European Commission should in future play a stronger part in supporting basic research (see page 734), but that may be detrimental to wider collaboration. How are those desirable goals to be balanced?

■ **Applied research.** The EEC centrally supports research intended to put its industry in better shape, but there are contradictions, not least that with the principle that successful industrial companies stand on their own feet, paying for their own research and development. EEC practice is also a potential impediment to wider economic collaboration. How will those issues be resolved?

In all this, there is a crucial issue too often overlooked — the danger that European collaboration in some defined framework will be exclusive of the world outside. The danger that the EEC will emerge from the upheaval planned for 1992 as a high-tariff chauvinistic group of self-satisfied states is the most immediate. Those who would suffer most would be those stranded within the tariff barriers, but Europe's relationship with the outside world would also be seriously damaged. That is why the EEC's goal of a single market, promised for 1992, is not in itself sufficient. It also matters crucially whether it is an out-going grouping. □



# Once-lame duck now exudes self-confidence

## Paris

THE European Space Agency (ESA) is "the best example of what European collaboration can achieve", says its director-general, Reimar Lüst. "We are not only involved with basic science and new technology, but are faced with heavy competition, unlike the European fundamental research facilities, which have life easy."

Yet, with only one-tenth of the budget available to the US National Aeronautics and Space Administration (NASA), ESA has become a force to be reckoned with.

The collaboration began in the early 1960s, under the impetus of European scientists, as the European Space Research Organisation (ESRO) and the European Launcher Development Organisation (ELDO). ESRO was highly successful, developing a series of scientific satellites launched by NASA, and establishing specialized space-science centres in the Netherlands, Italy and West Germany. But ELDO was a damp squib and failed to produce a viable launcher.

ESRO and ELDO metamorphosed into ESA in 1975 with the signing of a convention by 11 European countries: Belgium, Denmark, West Germany, France, Ireland, Italy, the Netherlands, Spain, Sweden, Switzerland and the United Kingdom. Austria and Norway joined last year, when Finland became an associate member.

ESA has taken European space capability out of a purely scientific domain, dependent on US launchers, into telecommunications, Earth observation and meteorology. A major step was the establishment, in 1980, of Arianespace, a commercial joint-stock company (59 per cent owned by France) responsible for the exploitation of ESA's successive generations of Ariane rockets. With a record of only four failures out of 30 launches, Arianespace now has 50 per cent of the world commercial launch market.

Once the heart of European collaboration in space, basic science now represents only 12.4 per cent of ESA's total 1,573 million ECU budget. But the science programme, like the general budget, is mandatory. All member states have to contribute in proportion to their gross national product. Consequently, unanimity is required whenever policy or budget decisions are put to the vote at ministerial-level meetings. Last year,

Britain found itself isolated when it initially refused to agree a 5 per cent annual increase in the science programme budget, holding up scheduling and potentially threatening some projects.

The science programme is constructed around a long-term plan, put together by the science programme committee and covering the next 15 to 20 years. The current programme, called Horizon 2000, was jubilantly approved at the 1985 ESA council meeting at ministerial level, in Rome. It comprises four 'cornerstone' projects in solar terrestrial physics, a comet nucleus sample return mission, an X-ray multi-mirror mission and a sub-millimetre astronomy project.

For Lüst, the science projects often help the push towards new technology — as was the case with the optics of the Hipparcos astronomy satellite, one of

adequate infrastructure.

But ESA's most controversial and expensive move, where tangible returns are less certain, came in November 1987 when a majority of member states backed a programme for manned space missions. Together, ESA's contribution of an attached laboratory to the NASA Freedom space station, a free-flying laboratory and a polar platform, now account for almost 40 per cent (609 million ECU) of its total budget, while the French-conceived Hermes space plane and the Ariane 5 rocket needed to launch it swallow a further 10 per cent (153 million ECU). Britain has opted out of these projects, so British companies will benefit hardly at all from the contracts.

Lüst applauds the decision to enter the manned space arena. "The trend will move towards robotics, but this will take 20–30 years. Meanwhile, there is still a need for people." He argues that the Hubble Space Telescope, due to be launched at the end of the year, has been designed to last for ten years. But if it needs repair during that time, "it may be more expensive to recover than to repair it in orbit."

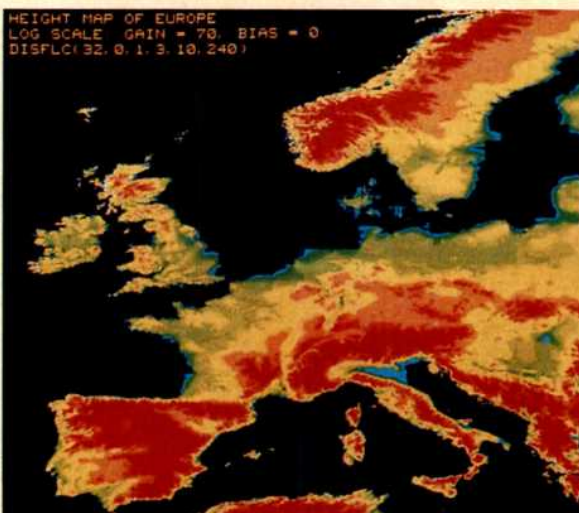
To Lüst, there is no doubt about the value of ESA for European science and industry. "The system really works. On satellite projects, probably two dozen truly European teams work together. Europe needs a drive on high technology and ESA provides an excellent vehicle for this." He is proud that ESA scientists and engineers are already exploring new frontiers such as robotics, synthetic radar and laser applications for optical communication between satellites.

"We also need more civil and less military research", says Lüst. Since 1980, the ESA convention has declared the purpose of its activities as exclusively peaceful, a constraint that has strung out negotiations with NASA over the Freedom space station. He also feels that space science is good value for money. "Europe's gross national product is about three-quarters of that of the United States, yet we spend only one-eighth per capita of what the United States spends on civil research. Only 0.05 per cent goes on space technology. Even if the figure doubled it would still be all right."

But last year, ESA found its member states less united and resolute than in 1985 and is due for a new round of debate when it seeks backing for the Data Relay Satellite, which, according to Lüst, is an essential element of the Columbus package.

Meanwhile, Columbus is not yet out of the woods. President Bush has still to persuade Congress to go ahead with Freedom. But, says Lüst, if the United States reneges, ESA will continue alone, although it may be more reluctant to cooperate with NASA in the future.

Peter Coles



ESA's satellite-view of Europe. Europe could comfortably double spending on space, says ESA's director-general.

four 'medium-sized' missions planned under Horizon 2000. (Hipparcos is due to be launched in a few weeks.) Two others, Ulysses and the Space Telescope, are joint ventures with NASA, and have had to stay on ice for almost three years since the suspension of shuttle launches in 1986. Future ESA missions will be launched by its own Ariane rockets.

The applications programmes are where national passions, purses and fantasies find direct expression. Participation is optional, but ESA ensures that backing nations receive industrial contracts in proportion to their investment.

Telecommunications satellites have been supported by all member states and, with Earth observation and microgravity projects, have stimulated the European Commission to become involved to work out norms, organize networks of users and to improve what it considers to be an in-



## EUROPEAN SOUTHERN OBSERVATORY

# Southern sky surveyed

## Garching, near Munich

CREATED out of the ruins of European astronomy after the Second World War with the help of the Ford Foundation, the European Southern Observatory (ESO) has become one of the most successful undertakings in European science. After years of solid contributions to optical astronomy, ESO now stands poised to seize the initiative internationally with its planned Very Large Telescope (VLT).

Following the model of the European nuclear research centre CERN, ESO goes beyond the financial abilities of individual member states to build powerful instrumentation in its field. Since the creation of ESO in 1962, its main tasks have been to build telescopes for scanning the Southern Hemisphere and to develop new instrumentation for optical astronomy.

Among telescope designers, ESO is now known as the pioneer of what are called active optics, embodied in its New Technology Telescope (NTT), which began a series of trial observations on a mountaintop in Chile in March. Active optics are the computer engineers' alternative to the cumbersome slabs of glass from which telescope mirrors have traditionally been made. Many hold that the diameter of conventional glass mirrors has now reached its technical limit, and that active optics are the only way forward.

The idea is that a microcomputer controls transducers which in turn control the precise figure of a thin glass mirror, distorted though that may be by gravity and other influences. The aim is superior resolution, but also telescopes that are more manageable and less costly. Active optics will play a pivotal role in the VLT, which is actually a set of four 8-m mirrors yielding the equivalent of a single 16-m dish. The telescope will be the largest in the world when it is completed in 1998.

The five original member states — Sweden, West Germany, Belgium, the Netherlands and France — were joined by Denmark in 1967 and Italy and Switzerland in 1982. Austria is now making a strong bid for membership. But despite an early interest, Britain has stayed out of ESO, concentrating at first on collaborations at observatories in South Africa and Australia and, more recently, on telescopes of which it is the chief owner in the Canary Islands and Hawaii.

ESO has its European headquarters in a smart modern building in the Bavarian flatlands at Garching, near the Max Planck Institute (MPI) for Physics and Astrophysics, the MPI for Plasma Physics and the campus of the Technical University of Munich. It operates or shares the 15 telescopes (counting the NTT) at La Silla in northern Chile, 600 km north of Santiago. The staff of 280 is divided

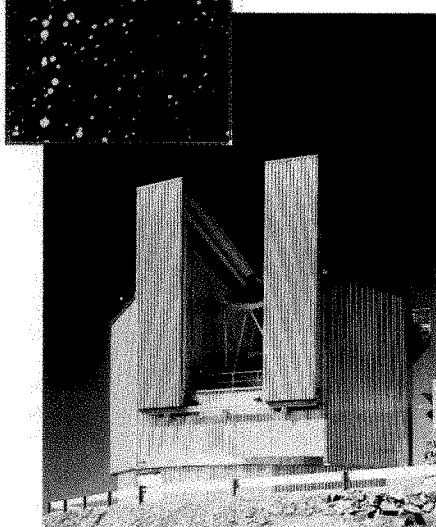
between Garching and La Silla, where most of the technical staff is Chilean. In Garching, there are about 50 astronomers (10 permanent) and 80 other staff members. The remote site at La Silla — Spanish for "the saddle" — in the Atacama Desert is "one of the most unpolluted sites on Earth in every sense", says Richard West, an ESO astronomer. The first telescope went into operation in 1968.

The organization of ESO is virtually identical to that of CERN (see page 722). An administrative council meets twice a year to decide budget and policy issues. A director-general (now Harry van der Laan of the Netherlands) is responsible for day-to-day operations. The scale is the most important difference: ESO is about ten times smaller than CERN. The annual budget of about DM50 million (it will rise to DM100 million in the 1990s because of the VLT) is shared by member states, with the larger states paying more.

ESO has generated excitement among European and outside astronomers with its ambitious plans for the VLT. Expatriate Europeans such as Jacques Beckers of the Netherlands, recently of the US National Optical Astronomy Observatory, have been lured back from the United States to work on the VLT, whose construction was approved unanimously by ESO member states in 1987. The VLT construction budget is DM382.2 million.

VLT is the logical offspring of NTT. The active optics of NTT are expected to allow the 3.58-m telescope a threefold improvement in resolution over that of previous instruments. Active optics will be a necessity for the VLT, whose 8-m mirrors would collapse under their own weight if ground at conventional thick-

The New Technology Telescope and results of 'first light' on 23 March. Inset shows an area near Omega Centauri, with 'seeing' low at 0.39 arcsec.



ness. ESO researchers expect to choose a site in Chile for the VLT by 1990. The first telescope may be working as early as 1995.

One of ESO's obvious difficulties is its popularity. As at other well-equipped observatories, only about a half of the 700-750 applications for observing time can be granted each year, and many successful applicants receive less time than they ask for. A committee made up of astronomers from each member state rates the proposals numerically, but the director-general has the final say. Van der Laan's task is "tricky and difficult", says West, especially because not all heavenly objects can be observed all the time.

Although ESO is a European organization, astronomers from member states enjoy no advantage in the refereeing process. Chilean, US and British groups have all been granted time in recent years. West says that the competition for time has raised the quality of European proposals — and, in the end, Europeans do receive most of the observing time.

What advantages accrue to ESO's member states? The most obvious is the organization's reason for existence — that astronomers have access to telescopes of a quality that national organizations would be unable or unwilling to finance. There are other tangible benefits, not least access to technical knowhow. Thus ESO was early in the field with the use of image-processing systems for extracting information from images of the heavens. Astronomers who used to travel to Garching to use its image-processing systems have often been able to find the funds with which to build their own, but ESO continues to provide software support to member states and others free of charge.

Italy and Denmark have decided to build versions of ESO instruments for their national astronomy programmes. Indeed, Italy joined ESO in part to gain the technology to build its own 4-m telescope. The project has a name — Galileo — but not yet a site.

ESO's small staff of professional astronomers, deliberately kept small, is regarded as a collective benefit. To keep ideas flowing in, there are only a few permanent positions. In striking a balance between a self-standing research organization and a service organization for its members, ESO (prodded by its council) has leant in the second direction.

ESO wins praise from astronomers not only for providing a unique opportunity for them to observe the southern sky. La Silla and Garching are also well known as meeting places where collaborations are incubated. VLT may be such a powerful magnet for observers everywhere in Europe that its influence could extend far beyond mere time-sharing. After all, as Beckers says, VLT "is where [optical] astronomy is going." **Steven Dickman**

## Fight for tape recorders

### Dwingeloo (Netherlands)

IMPROBABLY hidden in the pine-woods south of this hamlet near Groenningen is a radiotelescope and a smattering of low-built offices. The site is that of a European collaboration of radioastronomers who may yet be able to build a gigantic tape-recorder allowing a score of observatories elsewhere in Europe to pool their signals in the astronomers' common cause of seeing further. But the cause has been cruelly frustrated.

Dwingeloo is also the headquarters of the European Consortium for VLBI (for Very-Long-Baseline Interferometry). The technique of correlating accurately timed signals from several radiotelescopes, all pointing in the same direction, makes the accuracy of the position of a simultaneously observed radio source greater than the sum (in any sense) of the accuracy of the separate observations. The headquarters of the Netherlands Foundation for Radio Astronomy (NFRA) is also sited at Dwingeloo.

The European Consortium is, just now, at its wits' end because of uncertainty over its plans for the future. Last year, it applied to the Stimulation programme of the EEC for funds to finance its technical development. But the EEC Commission said "No".

In reality, the VLBI Consortium is a club. The founder members are the observatories at Bologna, Effelsburg (near Bonn), Dwingeloo, Jodrell Bank and Onsala (Sweden), and associate members include the Paris Observatory (Meudon) and institutes in Moscow and Poland. The six regular collaborators have committed between a quarter and a third of their observing time to VLBI. Projects are put forward by astronomers individually, and assessed by a programme committee. Richard Schilizzi, project manager for NFRA at Dwingeloo, says that the consortium is two to three times oversubscribed on a 24 hours a day basis. Moreover, the network is growing rapidly.

Like all VLBI collaborations, the club's collective ambition is so to improve on the accuracy with which measurements of the position of distant radio sources are made that the large-scale yardstick of the structure of the Universe will be better known, nearby galactic and even stellar phenomena will be better understood and the year-by-year movement of the Earth's tectonic plates will be more accurately measured.

The European Consortium is anxious not to be left behind by its competitors, the chief of which is that in the United States. Europeans are envious of the US VLBA (Very-Long-Baseline Array) project, which is now building ten 25-m dedicated radiotelescopes operating in

nine frequency bands between 1 metre and a few millimetres.

From the outset, VLBI techniques have been especially valuable in mapping the structure of distant quasars and other active galaxies; Schilizzi says the recent observation by VLBI of extragalactic water and hydroxyl masers offers a chance of constructing an independent and objective distance scale for the Universe.

So, with all that promise, why are people so glum? The difficulty is that the analysis of VLBI data is becoming steadily more complex — and expensive. Collaboration requires that each observatory should have a decent clock and a more than decent tape-recorder.

With the development of millimetre-wavelength telescopes (which improves the resolution of the system), recorders must be capable of storing huge amounts of data — 512 megabits a second is the new standard, which is roughly the information content of 100 standard video signals. But then it is necessary centrally to correlate up to 20 of these recorded signals electronically, which means synchronizing and then multiplying together each pair of signals from the contributing observatories.

The 18 million ECU for which the European Consortium applied last year would have been spent on the development of the tape-recorders and the correlator followed by their installation at Dwingeloo. The consortium points out that the only correlator in Europe at present capable of dealing with millimetre-wavelength signals is that at Effelsburg, which can handle signals from only three independent telescopes and which is, in any case, fully extended.

Schilizzi says that it is essential that the project should cover the cost of providing each of 11 telescopes with a tape-recorder (and a spare). Otherwise, he says, national grant-making agencies would skimp on the cost, and the resulting non-standard equipment would yield signals that could not easily be compared.

There is a Euro-industry argument on the consortium's side: broad-band recording techniques and equipment will be in great demand as high-definition television gets under way. So why not put some millions of ECUs into the development of such machines, equip a score of observatories with them more cheaply than they could be bought and leave some European manufacturer with an expertise now lacking?

It seems generally agreed, even in Brussels, that the argument is appealing. People have plainly listened to it attentively — and have then decided that there is nothing they can do. But the last has not been heard of Dwingeloo's ambitions. □

## Monastic calm and money worries

### Paris

JUST a 20-minute train journey from the centre of Paris in 11 hectares of parkland lies what has been described by its director, Marcel Berger, as "a monastery and a beehive" — the internationally renowned Institut des Hautes Etudes Scientifiques (IHES). The paradoxical metaphor is justified.

On the one hand, the institute provides the isolated calm necessary for profound meditation on theoretical problems, while the six permanent fellows last year attracted 872 researchers from 42 countries to exchange ideas in the vanguard of mathematics and theoretical physics.

Inspired by the Institute for Advanced Study at Princeton, IHES was set up in 1958 by Léon Motchane, Jean Dieudonné and Robert Oppenheimer as a European centre of excellence in mathematics and theoretical physics. The Second World War had seen some of Europe's most able scientists seek refuge in the United States; naturally, there were fears that the centre of gravity of science would move irretrievably across the Atlantic. In poetic justice, IHES stands on the site of a chateau commandeered by the Nazis during the occupation.

Very much like its Princeton model, IHES has a small number of permanent fellows on comfortable stipends for life — although mathematician René Thom is the first to continue to retirement so far, becoming an honorary fellow this year.

Fellows are free from all teaching and administrative obligations and do not have to account for their time, apart from an undertaking to spend 6 months of the year at the institute. In practice, they have catalysed revolutions in their specialisms. During the 1960s and 1970s, for example, the institute established itself as the world's temple of algebraic geometry, through Alexandre Grothendieck, Jean Dieudonné and Pierre Deligne.

Although fellows leave an indelible mark on the choice of themes, these have evolved over the years, aided by the presence of a handful of invited long-term visitors who stay for around five years, and a long line of visitors spending up to a year at the institute.

Today, the permanent fellows are Jean Bourgain, Mikhael Gromov, Louis Michel, David Ruelle and Dennis Sullivan. This year, theoretical physicist Thibault Damour fills a vacancy left by Oscar Lanford, opening a new avenue of research in general relativity and cosmology.

The institute's private status is anomalous, for 64 per cent of its FF19,287,000

## SYNCHROTRON RADIATION

## Grenoble as physics city

## Paris

THE French city of Grenoble, near the Swiss and Italian borders, is, by a mixture of fate and design, becoming a 'Eurocity'. But unlike Brussels, Strasbourg or Luxembourg, Grenoble's cosmopolitanism stems not from politics but from a distinctive blend of unrivalled basic science facilities and new technology industry. The city's fourteenth-century university may already have had an international flavour, but twentieth-century physics has put Grenoble on the map of European science.

In 1946, the French national science research centre (CNRS) opened its first major laboratory outside Paris in the city, with its Service National des Champs Intenses. In 1956, the French atomic energy commission (CEA) chose Grenoble for its research establishment and in 1966 the Institut Laue-Langevin, which houses the world's highest-performance neutron-beam reactor, joined the family.

This year, construction has started on the latest, and most European, of Grenoble's big science instruments — the European Synchrotron Radiation Facility (ESRF). The idea that Europe should build its own major synchrotron facility was first put to the European Science Foundation in 1975. There followed a period of diplomacy, definition and costing until, in 1985, the two major promoters, France and West Germany, proposed to go ahead with the project.

At the end of 1985, a memorandum of understanding was drawn up between France, Germany, Great Britain, Italy and Spain. In 1987 the protocol was signed, with Switzerland and a Nordic consortium joining the original five members. The formal convention and statutes allowing money to be spent was signed in Paris on 16 December last year.

At present, the site is being excavated, but, says Karl Witte, assistant to ESRF's director (Ruprecht Haensel), "you can already see a few circles on the ground". The first seven of ESRF's beam lines are due to be opened in 1994, with a target date of 1998 for the full 30 planned. When completed, ESRF will be the world's most powerful radiation synchrotron, able to accelerate electrons or positrons up to 6 GeV, with a beam width of about one tenth of a millimetre.

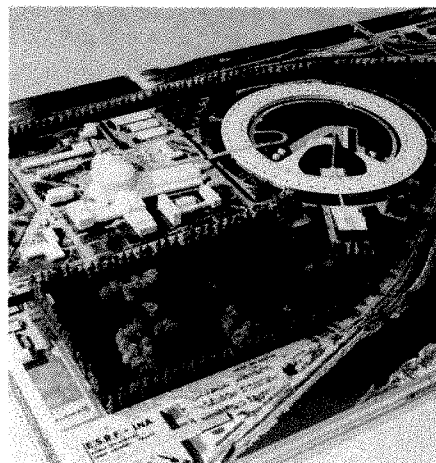
But the title may quickly be lost. A 7 GeV synchrotron is to be built at the Argonne National Laboratory in Illinois and, last December, the Japanese government announced plans for a 8 GeV synchrotron facility near Osaka.

The total cost of ESRF will be FF2,600 million (about \$406 million), with operating costs for 1994–98 estimated at FF1,000 million. France is contributing 34 per cent of the cost, and in addition has

supplied the site and the local infrastructure. West Germany is the second largest partner, with a 24 per cent stake, followed by Italy (14.5 per cent) and Britain (12.5 per cent). The balance will be shared between Spain, Switzerland and the Nordic countries (4 per cent each) with Belgium providing 3 per cent.

Last month, at the first ESRF users' meeting, 200 Europeans and a handful of colleagues from the United States, Japan and Israel, discussed priorities for the first seven beam lines and verified that the synchrotron's specification (called the 'red book') is still appropriate. Discussions will continue until the scientific advisory committee meeting at the end of June.

Although ESRF still has only a skeleton staff, there are already signs that it may be difficult to achieve a mix of nationalities in the resident staff (on five-year fixed con-



A model institute, but building is under way.

tracts) which reflects members' relative contributions. "We wanted to keep the French presence below 50 per cent", says Witte, but the proportion seems now to be about 52 per cent. The difficulty is that it is not easy to attract scientists and technicians from other member states because ESRF can offer only French salaries in line with those of the CEA. Constitutionally, ESRF is a French company and in that respect unlike, for example, CERN, where salaries are fixed on the international European scale.

More than 2,000 people are expected to use ESRF each year, with all costs paid for out of member states' contributions. Experiments will be selected on the basis of merit by a scientific panel on which each member state will be represented. If a "significant imbalance" emerges between a country's rate of use of the facility and its percentage contribution, the statutes provide for an eventual retrospective "readjustment" of payments. This principle may free referees assessing proposals for using the machine from the straitjacket of national time-sharing quotas.

P.C.

(\$3.2 million) budget comes from the French Ministry of Education — an initiative started by President Georges Pompidou in 1965. Before that, IHES scraped along with relatively small subscriptions from a handful of business sponsors. But this year, only FF570,000 (\$95,000) has been contributed by the private sector. Now, laments Marcel Berger, it is almost impossible to find money for basic research in industry. This has always depended on personal contacts established by the incumbent president, and can lapse when there is a change at the top.

Although genuinely international, IHES derives only 15 per cent of its income from European governments and is not eligible for European Commission support. Since 1971, the British Science and Engineering Research Council (SERC) and the West German Max Planck Gesellschaft have each given grants, this year of just over FF1 million (\$166,000). The Swiss and Belgian governments have also supported IHES since 1971, together providing about 5 per cent of its income, while the governments of Ireland, Denmark, the Netherlands, Finland, Portugal, Israel and Brazil also make modest contributions (between \$2,000 and \$13,000). The US National Science Foundation (NSF) has supported IHES since 1985, this year giving \$65,000. This grant, a rare gesture for NSF, reflects the large number of US visitors to IHES, by far the majority.

But money is becoming an increasing worry for the institute, which started the year in deficit. "The private status of IHES gives us great flexibility and autonomy", says Marcel Berger. "The fellows' stipends, while they cannot match those available in the United States, aim to free them from money worries, while it is possible to organize a seminar or arrange a visit at the drop of a hat."

But, with the library — a former music room — cracking under the weight of its books and needing expensive underpinning, Berger is having to buy time by leaving vacancies unfilled. An important ally has been found in the European Science Foundation which, since 1976, has carried out a five-yearly review of the IHES scientific programme. The panel's review helps guarantee the institute's autonomy, while ensuring that its high standards and international connections are maintained. That, in turn, makes governments — and, in principle, industry — happier to continue their support.

Berger says it is "unthinkable" that IHES would ever be threatened with closure. But if any of its sponsors, such as SERC, should be prevented by national government policy from helping, would suffer and its sometimes fragile occupants could once again become an endangered species in Europe.

P.C.



# Europe's most influential and costly collaboration

## Geneva

CERN, the European Laboratory for Particle Physics whose new name does not match its old acronym, seems always to be celebrating something. A few years ago, it was the successful production and identification of the particles called the  $Z^0$  and the  $W^\pm$  whose existence put the cap on the unified theory of electromagnetic and weak nuclear forces due severally to Salam and Weinberg.

This year, CERN will be celebrating the commissioning of its latest accelerator, called LEP (for large electron-positron storage ring).

With just a few months to go to completion, LEP seems already assured of success. The tunnel (with a circumference of 27 km) has long since been complete and the magnets which keep the two counter-circulating beams installed. One section of the storage ring has been tested for fidelity with real electrons. The hope now is that it will be possible to put electrons and positrons into circulation soon after midsummer and that the first physics will be done early in the autumn.

The most striking feature of the way that people talk about this prospect is their confidence. The underlying assumption is that every project to build a particle accelerator that CERN touches turns out successfully. Certainly this has been the laboratory's record since the late J.B. Adams, as director, built the machine called the Super-Proton Synchrotron (SPS) and began on the task of building LEP.

The immediate objective is to collide beams of electrons and positrons circulating in opposite directions in the same vacuum chamber and each carrying 55 GeV of energy. Apart from the gigantic scale of the construction, the technical difficulties are those of shaping the counter-circulating beams of electrons and positrons and controlling the position of the particles both in space and time. The particles travel in bunches and are

meant to coincide in space and time at the eight points around the ring at which collisions are meant to take place.

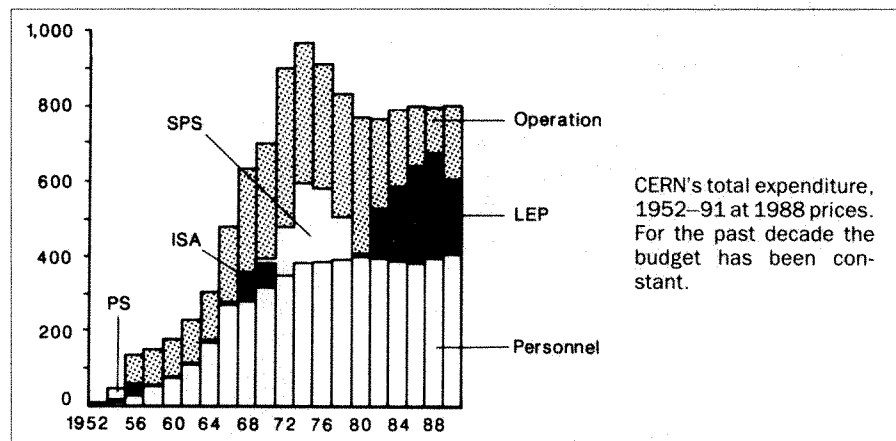
With this energy, colliding pairs of electrons and positrons should yield copious numbers of the heavy bosons required by the electroweak theory, allowing the properties of these particles to be defined in detail. But there is also a scheme for making a more powerful machine of LEP by substituting superconducting cavity oscillators for those now meant to supply LEP with power. The result would be, perhaps by 1995, a machine capable of colliding 100 GeV electrons and positrons.

The scale of this project is quite breathtaking. The surface of the site shows no sign of the storage ring, which is in places

be a formal examination of continued British membership of the international high-energy physics community (carried out by a group under Sir John Kendrew), a further examination under the eye of the CERN council of the efficiency of its operations and, finally, a decision that British membership would continue, at least for the time being.

It is understandable that the workforce at Geneva (about 3,500 strong) should feel slighted by these signs of less than full approval. They are quick to point out that, as high-energy physics laboratories go, CERN is cheap, with a budget of just over 800 million Swiss francs a year out of which it must pay for the physical cost of building LEP (roughly SFr1,200 million since construction began in 1981).

In reality (see figure), CERN has been required to live within a fixed budget since the beginning of this decade. In the process, it has become expert at cannibalizing its existing machines. Electrons and positrons



100 metres or so underground despite the way the ring has been tilted from the horizontal (to minimize excavation costs). Getting to a particular experimental hall requires a journey in a vehicle through the rural lanes of (mostly) France and Switzerland, where agriculture continues undisturbed.

The hall that houses the planned experiment called Delphi is really an underground cavern 30 metres high which, last month, was buzzing with people threading power and data cables through the proper positions in the harnesses designed for them as if they were acrobats building a cocoon of cable for a mechanical monster lost underground (see this week's cover). Half-way up the detector being assembled are the ports that will deliver coincident bunches of electrons and positrons simultaneously to the centre of the detector.

That is the heroic face of CERN, which is also, by being the most costly of all international collaboration in Europe, also one of the most controversial. More accurately, that has been how it has seemed from Britain in the past few years.

In 1984, it was decided that there should

for LEP, for example, will be given their high energy by first circulating them through the oldest machine in operation at the site (a proton synchrotron) and then through the SPS (giving them a total energy of 20 GeV) before they are fed into the larger LEP ring for final acceleration and storage.


A bizarre prospect for the more distant future is that, at some stage, the same cascade of accelerators may also be used for the purpose for which they were designed (accelerating protons or anti-protons) with which to fill a companion hadron storage ring in the same LEP tunnel. Since the two booster synchrotrons also have other functions on the site, the planning of what kinds of particles are delivered to which experimental rigs in what sequence threatens to become a planners' nightmare.

The Large Hadron Collider, the scheme for equipping the LEP tunnel with a second circular vacuum chamber for storing counter-circulating protons and antiprotons, is for the time being only a possibility. For one thing, the 14 full member states have not approved the scheme (which, with its superconducting

## Science in Europe

THIS brief survey of collaborative research in Western Europe has been compiled by Steven Dickman (Munich Correspondent), Peter Coles (Paris Correspondent), Peter Newmark (Deputy Editor), whose contributions are attributed, and John Maddox (Editor).

The objective has been to illustrate by means of examples the manner in which collaborative research in Europe is undertaken rather than to provide a comprehensive survey of the institutions involved, and of their objectives. □



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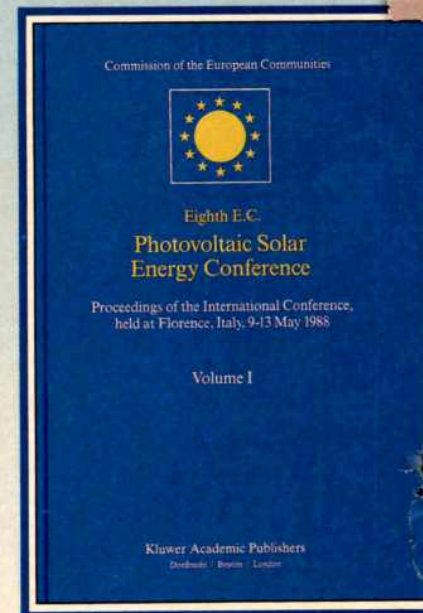
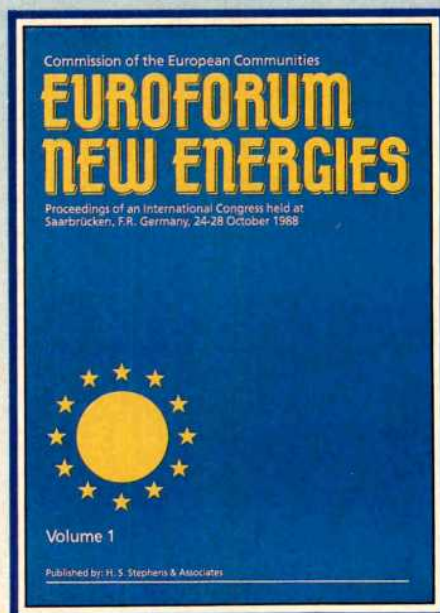
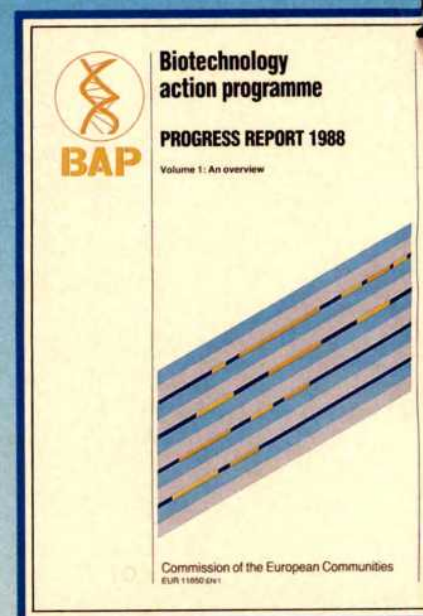
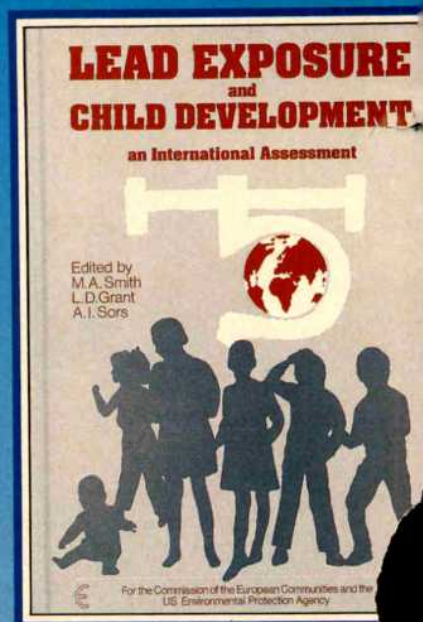
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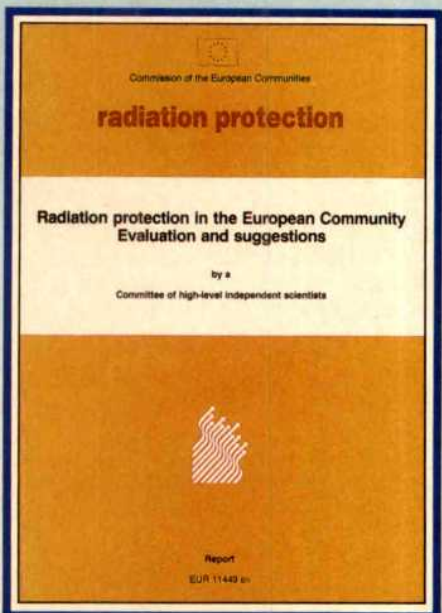
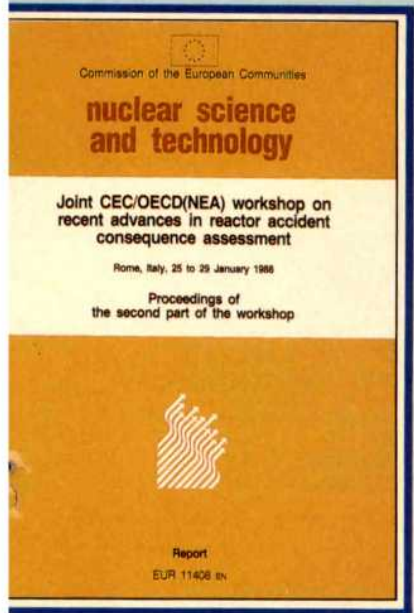
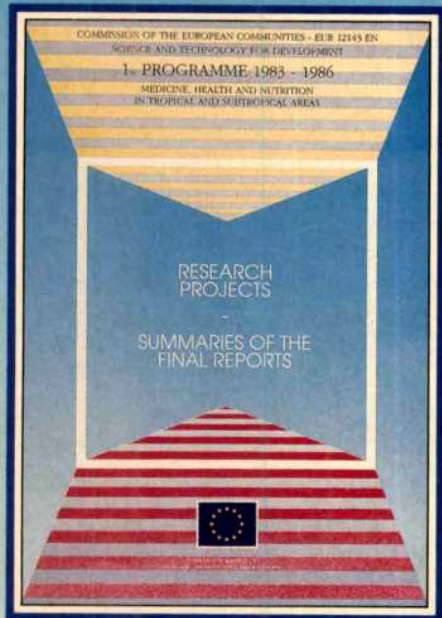
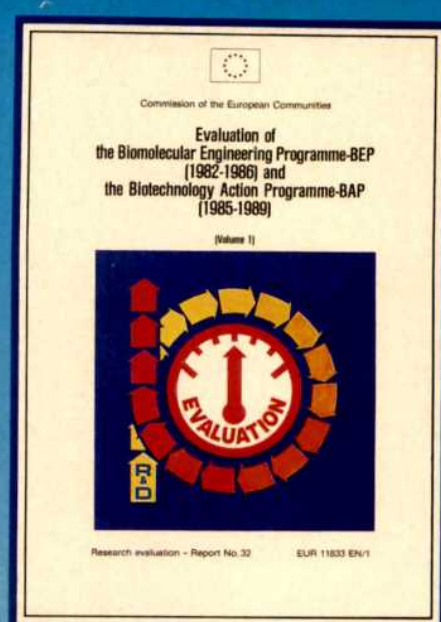
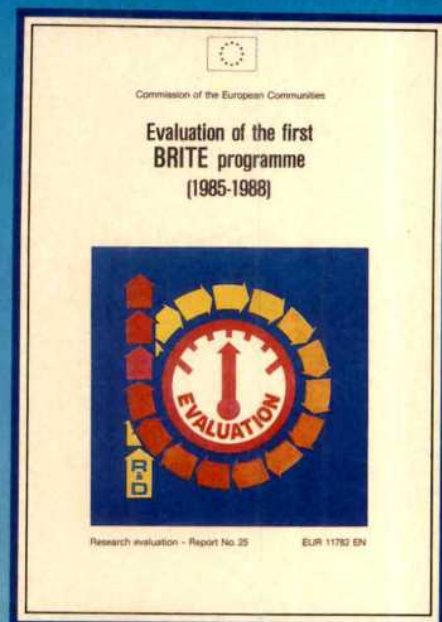
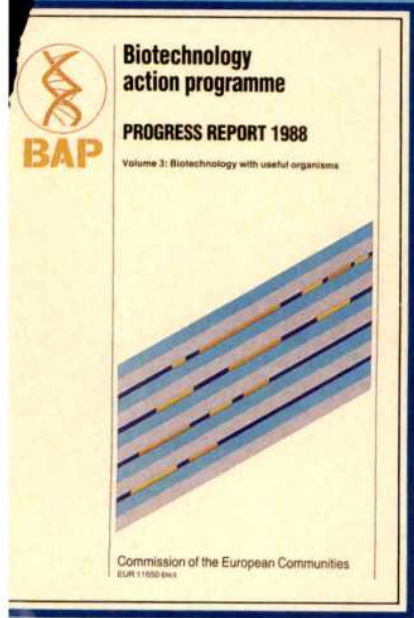




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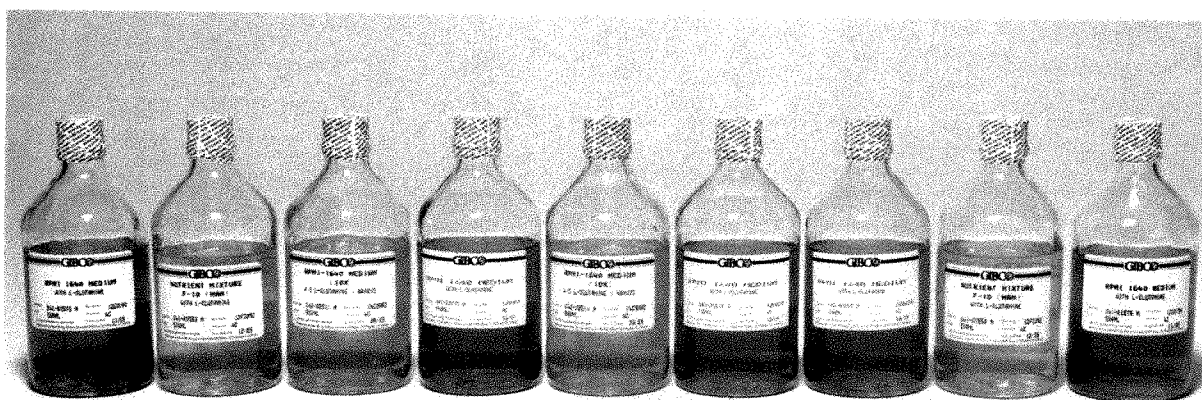






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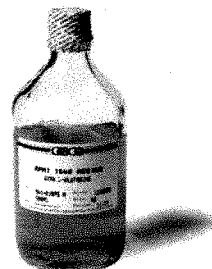
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ESF

## Small but a powerful membership

London

THE European Science Foundation (ESF), based in Strasbourg, is the smallest but one of the most interesting of all European collaborations. It has a budget of its own amounting to less than FF14 million a year, but its influence is much greater than its budget would normally command.

The explanation is that ESF's members are neither people nor governments, but public grant-making agencies in European states. From West Germany, for example, both the Max-Planck Gesellschaft and the Deutsche Forschungsgemeinschaft (DFG) belong. All five of the British research councils as well as the Royal Society are members. Altogether, there are 50 members from 18 European countries, Finland and Turkey included.

In practice, what this means is that a project which is backed in Strasbourg is likely to win a sympathetic hearing when its advocates later take the hat around asking for substantial funds to supplement whatever seed money that ESF may have provided.

Perhaps the most striking illustration of this successful way of working is the launching of the European Synchrotron Radiation facility, now being built at Grenoble (page 721). The project first surfaced about a decade ago as a recommendation of an ESF committee consisting of accelerator physicists and potential users.

ESF knew the cost would be beyond its scope, but reckoned that it could carry out the diplomacy required to build a consortium of its grant-making members to launch the project, which has now successfully been done.

Another ESF-originated programme is the European Geotraverse, a project for the geophysical investigation of the Earth's crust along a line from Scandinavia to north Africa.

Latterly, ESF has been spending much of its budget on supporting networks of research with funds with which to organize meetings and regular visits among themselves. At the same time, it appears to have accepted that the time has come to grow. ESF's director, economist Michael Posner, is working on a "forward look" that would multiply ESF's budget by three or even five.

The foundation has also appointed a new president to take over from Professor Eugen Seibold, whose term ends in 1990. Professor Umberto Colombo, chairman of Italy's Nuclear Energy Agency, will take over in November 1990. □

magnets, would cost "1.5 LEP").

In any case, physicists would prefer to see what LEP itself produces before linking their ambitions to a particular successor. Some choices, the possibility of building opposed electron and positron colliders, for example, might make it necessary to leave the cramped site at Geneva.

On the face of things, CERN can thus see the way forward until the end of the century, which will amount to roughly half a century after its foundation (at a meeting organized by UNESCO in 1951). What, by then will it have accomplished?

Judgements of this kind are necessarily subjective. From the start, CERN has been most notably a European citadel of technical expertise, both in the construction of scientific instruments on a huge scale and in the development of fast data networks and communications interfaces for the handling of the data it produces.

While there is a sense in which CERN would be a pale shadow of its present self

CARLO RUBBIA

## New machinery on the horizon?

Geneva

CARLO Rubbia is a tall man with a stoop who became director of CERN at the beginning of the year, after helping to put the institution on the map by finding the intermediate heavy bosons (and being awarded the Nobel prize for physics in 1987). He seems to have thrown himself into his new job with high-energy diplomacy, talking to the high-energy physics communities elsewhere, notably in the United States.

Rubbia is in no doubt that CERN is technically more excellent at the craft of building particle accelerators than either the United States or the Soviet Union. He points to the high estimated cost of building the SCSC in Texas, probably in excess of \$4,500 million, and to the difficulties (now resolved) of grafting onto the Stanford Linear Accelerator a device for colliding electrons at high energy.

But Rubbia also acknowledges that accelerators cannot keep on growing as they have been, and that the time must come when accelerators are built in a wider framework than in the past. Whence the diplomacy.

The strategy is to keep CERN in the forefront of high-energy physics while arranging that member states in Europe do not have to meet all the extra cost. Rubbia claims that Geneva has the world's only permanent team of accelerator designers and builders whose success has been established by demonstration. Will not others wish to join in collaborations with them, not just for physics but for construction?

One intriguing possibility is that future members in large consortia might make contributions in kind to future projects. There is even talk that the Soviet high-energy physics laboratory at Serpukhov,

had it not been for the discovery of the  $Z^0$  and the  $W^\pm$  mesons, that slights the importance of the growing range of general physics that pours out of Geneva each year, from the precise determination of the gyromagnetic ratio to the fabrication of atoms of antimatter.

Luck has helped. At the outset, with nuclear physics riding high and not easily distinguished from high-energy physics, CERN enjoyed the enthusiastic backing of powerful figures in Britain, France, West Germany and Italy. But by being consistently more cosmopolitan than its membership (with deliberate policies of forging links with Soviet and US physics), it has won and kept a place in international high-energy physics. In the process, it has become a free-standing centre of excellence whose connections run through the whole of Europe. There are many projects on which Europe spends 800 million francs a year without anything like the benefit. □

which is already fabricating superconducting magnets for its own new proton accelerator, might carry on making magnets for the Large Hadron Collider at CERN when its own needs have been met. Rubbia will make partnerships with anybody, on any favourable terms, to see the next generation under way.

But what would happen CERN if good sense required that the next machine should be built, perhaps by a larger consor-



Rubbia stresses CERN's ability to build accelerators.

tium, elsewhere? Rubbia notes that the present machines will have life left in them at the turn of the century, and that diversifying of present interests could also provide continuity on the Geneva site. But that is Rubbia's cautious side. The chances that he will be making a fierce fight for some new machine at Geneva a few years from now. □



# One of the better models of European cooperation

## Heidelberg

LENNART Philipson, an imposing Swede, is particularly proud of 'The Operon', the new 320-seat auditorium at the European Molecular Biology Laboratory (EMBL). It symbolizes his drive, as director general of EMBL since 1982, to fulfil one of the original aims of the 15-year-old laboratory — that it should be a European centre for teaching and training in molecular biology, particularly for the benefit of the smaller countries that help pay the cost.

Training, however, was only one of the main roles foreseen for EMBL by those who argued 20 years ago for its foundation. They also claimed it would act as a meeting-place for senior European molecular biologists (as Cold Spring Harbor does for US biologists and CERN for European particle physicists), a provider of specialized facilities that would be beyond the means of most European countries (again like CERN), and a focus for molecular biology that could help to disseminate the techniques throughout Europe. Critics at the time argued that specialized facilities and large laboratories would funnel molecular biology and its practitioners into an elitist group of little influence on the development of the science in Europe.

With its training role to the foremost, EMBL now hosts annually a dozen practical training courses, most of them run by its own staff, and takes on about ten pre-doctoral students, whose quality is said to be exceptional. They take their places alongside the 120 or so postdocs who are present at any time, in a laboratory that is demonstrably European to judge by the mix of nationalities of the scientific staff.

But Philipson argues that the training functions of EMBL are much more extensive. With almost all the scientists employed on short- or medium-term contracts, there is no elitist group. Rather, youngish scientists are given an opportunity to make their scientific mark during their most productive years, before dispersing, often to their country of origin, as trained leaders with a European attitude.

The other side of that coin is less bright: with such a turnover of even its better-known scientific staff, EMBL has some difficulty in building up and sustaining a reputation as an outstanding laboratory, which is what many of its critics think it should be for its size — about 500 people, of whom half are on the laboratory staff — and cost — now about £17 million a year.

At times, this has led one or other of the countries that foot the bill for EMBL to threaten to withdraw. The biggest threat, which ran throughout 1983, came from Britain, but ended when a Medical

Research Council review of the laboratory concluded that the British contribution could not have purchased equivalent work of equal quality within Britain.

In fact, no country has withdrawn its support from EMBL, and several more have joined in recent years, although Ireland, Iceland, Portugal and Belgium remain unpersuaded. Of these, only the Belgian contribution would make a noticeable addition to EMBL's budget. If Belgium joined there would be funds to occupy the last remaining space at EMBL — a floor of the brand new wing that houses 'The Operon'.

The same wing already houses the Bio-computing Programme, which recently became the seventh research programme at EMBL and which is, in many ways, just the kind of activity in which a European laboratory should be engaged.

On the one hand, it is an attempt to build a substantial research programme in an area of science — theoretical and computational biology, particularly in relationship to proteins and nucleic acids — in which Europe needs urgently to invest. But that is why it is disconcerting that EMBL is finding serious difficulties in recruiting experienced group leaders.

On the other hand, the Biocomputing Programme includes the EMBL Data Library, which shares with its US and Japanese counterparts the unenviable tasks of feeding emerging DNA sequences (27 million bases so far) into a database and then making it accessible worldwide. The former task is being eased because sequences are increasingly being deposited directly in the library, and the latter by the advent of EMBnet, which will be able to provide access to the current library through national European computer nodes on a DecNet-based network.

Even so, the accelerating rate at which new DNA sequences are being produced means that EMBL will not for long be able to support or even house the data library. Already, the European Commission supports 40 per cent of the activity, but only for two years in the first place, and Philipson is pushing for the foundation of a separate European institute (of bio-informatics, or some such) eventually to handle the library and its functions.

Of EMBL's other research programmes, those known as Gene Expression, Differentiation and, especially, Cell Biology have established reasonable international reputations. The Biological Structures programme, while still struggling to achieve equal footing, is said to be in better shape than it has been.

Meanwhile, the Physical Instrumentation and Biochemical Instrumentation

programmes are in danger of disappearing as a matter of policy. Stemming from the original conception of EMBL as molecular biology's counterpart to CERN, the development of instruments has always been one of its missions. Pursued somewhat in a vacuum by the founding director, (now Sir) John Kendrew, instrument development has been much more closely tied to biological programmes by Philipson, who now plans to integrate them completely.

This plan, like other major policy changes at EMBL, is subject to the approval of the laboratory's governing body, the council, which comprises delegates from each contributing country and is advised by a scientific committee. As director general, Philipson is responsible for planning and execution of approved scientific programmes at EMBL, assisted by a committee of staff members.

EMBL also operates two outstations, both with an uncertain future. For that at the Institut Laue Langevin in Grenoble (see page 723) the problem is that neutrons have not turned out to be particularly useful for biologists. Grenoble, however, is the site chosen for construction of ESRF (the European Synchrotron Radiation Facility, see page 723), which will be much more useful, and where EMBL has been asked to provide a biological research support laboratory. If EMBL agrees, the days (or, rather, years) of the second outstation at DESY (the Deutsches Elektronen-Synchrotron) in Hamburg will be numbered.

Until a few months ago, Philipson had not expected these problems to be his. But after the pathetic failure of the EMBL council to find a new director (see *Nature* 337, 397 and 589; 1989), he will stay until 1995 rather than leave in a year's time.

Perhaps for that reason, and tired of being on the defensive about EMBL as well as confident that the laboratory provides much better value for money than Community programmes, Philipson is now strongly promoting the idea of founding more EMBLs, for example, one devoted to plant biology and another to neurobiology.

Peter Newmark

Top men at EMBL (Philipson, left) and EMBO (Tooze)





EMBO

## Workshops to postdocs and publishing

Heldelburg

IN contrast with EMBL, it is hard to find anything but praise for its original parent, the European Molecular Biology Organization (EMBO), which distributes fellowships, finances workshops and practical courses and now produces the *EMBO Journal*.

EMBO celebrates its silver jubilee this year. Its first grant was a small one from Israel, which to this day qualifies as a European state both for EMBO and EMBL. That was followed by a substantial grant from the Volkswagen Foundation. Finally, in 1969, an intergovernmental agreement established the European Molecular Biology Conference, the body through which EMBO's (but not EMBL's) funds are still provided. With Portugal soon to join and Belgium contributing unofficially, EMBO has a full house, and an annual budget of nearly £4 million.

The membership is drawn, somewhat unselectively, from the ranks of European molecular biologists. But the keys to its success are the select committees and reviewers, who decide which applications to support, and John Tooze, EMBO's executive secretary. Tooze also manages to edit *EMBO Journal*, act as scientific co-ordinator for EMBL and carry out research (four papers in *J. Cell Biol.* in 1987).

The greatest pressure on EMBO's budget is for long-term fellowships supporting postdoctoral fellows for 1–2 years in a country other than their own. Demand for these fellowships has increased greatly in the past five years, but plans to meet it were somewhat thwarted by British penny-saving in 1986, when EMBO's existence was guaranteed until 1995 and its budget set until 1991.

As a result, only about 30 per cent of the new applications for long-term fellowships could be financed last year, while about 40 per cent deserved support, says Tooze. Nevertheless, 142 new awards were made, together with 69 renewals of one-year awards for a second year.

Awards, Tooze emphasizes, are made strictly according to the quality of the application. There is no attempt to distribute some of the awards to each of the countries contributing to EMBO's budget — last year, 40 per cent of the new long-term EMBO fellows were working in US laboratories.

Of the original plans for EMBO, only the idea that it should award research grants remains unfulfilled. There are those who believe the time is right to start pressing for EMBO to be allowed to build on its success in that way. **P.N.**

HUBERT CURIEN

## Portrait of a European

Paris

HUBERT Curien is a European enthusiast. Perhaps more than anyone, this modest 65-year-old French physicist, who is also research minister in the Socialist French government, has accompanied European science and technology in its trajectory from a collection of national initiatives towards true collaboration.

The European Science Foundation (ESF) and the European Space Agency (ESA), along with the European Commission's EUREKA and PACE programmes, CODEST and, most recently the Academia Europaea, have each benefited from his innovative flair and enthusiasm.

Although he has been president of both ESA and ESF, Curien was first of all an



Ministère de la Recherche et de la Technologie

Curien — urbane, optimistic and idealistic.

ambassador for French science through his research on crystals and even has a new mineral, 'curienite', named after him. He has been director of the French national science research centre (CNRS) and president of the French national space research centre (CNES). He became Minister for Research and Technology in the 1984 Fabius government until the 1986 elections brought the opposition conservative party to power. In June last year, he once again became Minister for Research and Technology in the new Rocard administration.

Looking back on the past 30 years, Curien acknowledges that personal contacts between scientists inspired the creation of the first European organizations. "European countries did not know each other well in the 1960s", he says. So it was as a result of informal contacts between colleagues such as Curien, Lord Flowers and Reimar Lüst that the European Science Foundation and ESA got off the ground. "Now", he says, "we know each other better" and there is a European synergy arising from the various organizations and forums that have been established.

Historically it was through the creation of joint large scientific instruments, such as CERN, that European scientists found they could buy their own cake and eat it,

without having to be guests at the table of US colleagues. Other examples have followed, including the European Southern Observatory and the European Synchrotron Radiation Facility. But for Curien, European collaboration in science and technology means more than sharing expensive facilities. It is also a matter of bringing scientists together, not only to exchange ideas, but to influence policy.

"If thinking comes from the bottom and the money from the top, people at the bottom find themselves in a very happy situation, and as it is the people at the bottom who do the work, it is better if they are heard", Curien said in his keynote address at the foundation of the European Academy last year. And this 'bottom up' approach is the hallmark of initiatives in which he has played a central role.

EUREKA, Curien's brainchild, provides for trans-frontier technology collaboration where the projects are market-led. Similarly, CODEST, the European Commission's (EC) Committee for European Development of Science and Technology, is a platform for specialists to influence policy, while the proposed EC Assembly, due to be debated this year, will give 200 academics a voice in Brussels.

Perhaps because his own roots are still in the laboratory, Curien knows that the whole is also composed of parts which can easily find themselves isolated. It was he who first proposed the idea of 'networks' of laboratories as an alternative to the 'lame-duck' idea of European centres of excellence. Since 1974, 20 networks have been set up in specific disciplines, largely through ESF.

Now, as Europe finds its stride, Curien sees a danger that countries such as Greece and Portugal will find it difficult to keep up. "European cohesion can easily be opposed to excellence", he says. In areas at the vanguard of science and technology, he says, poorer countries may lack the facilities or trained researchers to take part. But if Europe opts for cohesion and drops these projects, the best are brought down to the level of the least good. For Curien, one way to achieve cohesion and excellence is through education.

The EuroPACE satellite teaching programme for continuing education, of which he is president, is one means to achieve equality of skills. He also wants to see more part-time posts in universities to allow researchers from poorer countries to keep a foot in good laboratories while raising standards at home.

Urbane, optimistic and idealistic, Curien continues to seek ways in which European collaboration can improve. "We cannot imagine Europe continuing as it is now. It must be more coherent." But, he adds, "with patience you can always get what you want". **P.C.**

# What kind of bureaucracy is Brussels?

## Brussels

EUROPEAN science is scattered over a score of countries: why look for it in Brussels? Because the European Commission, the executive arm of the European Economic Community (EEC), is there and has an increasingly important if controversial influence on the shape of things.

But who wants European science to be run by a bureaucracy? The question is often asked, much to the distress of the Commission's staff, whose members believe "bureaucrat" to be a pejorative term. This brief constitutional history of the EEC may put the question in a broader context although without altogether removing the slur.

The Treaty of Rome, signed in 1957, on the model of the US Constitution, defines a kind of European government with three arms — an executive (or administration) which is the Commission, a supreme court (the European Court of Justice) and a legislature (the equivalent of the US Congress). But the likeness between Brussels and Washington is only formal, chiefly because the political equivalent in Europe of the US Congress is a bastard blend of representation by member governments and by the directly elected European Parliament (which is not in Brussels at all, but at Strasbourg or Luxembourg according to the season of the year).

Much of the long-standing and broad argument about the future of the EEC turns on questions of how the Commission should be controlled politically. At the outset, the Commission was the servant of the Council of Ministers, which for practical purposes is a committee of national ministers chosen (by the governments they represent) according to the nature of the issue to be decided — agriculture ministers decide agricultural issues; prime ministers or their equivalents (plus retinues) attend the biannual meetings of the council proper, organized by whichever country has held the chairmanship of the council for the elapsing half year.

Originally, the council and its sub-councils were expected to reach decisions by consensus, which meant that each national government could veto the wishes of its fellow-members. Now, under the European Single Act of 1986 (whose most important effect is to promise the operation of a genuinely common market by the end of 1992), consensus is required only on decisions of a largely constitutional character (including the extension of EEC membership).

The sphere of influence of the European Parliament is still evolving, but is

comparable with that of the House of Lords in the British constitution. It is asked to approve the EEC budget each year (and can delay its consent), is consulted about issues bound for a decision by the council, can raise specific issues (complaints at the effect of Commission decisions on member states, for example) and can draw up and approve reports on broad policy questions (as on environmental issues) which, although not binding on the Commission, are regarded seriously in Brussels.

European federalists naturally look forward to a time when the influence of the European Parliament will have so grown that national parliaments have no more than the importance of state legislatures in the United States. Others, and governments such as the British, have a different view of the future.

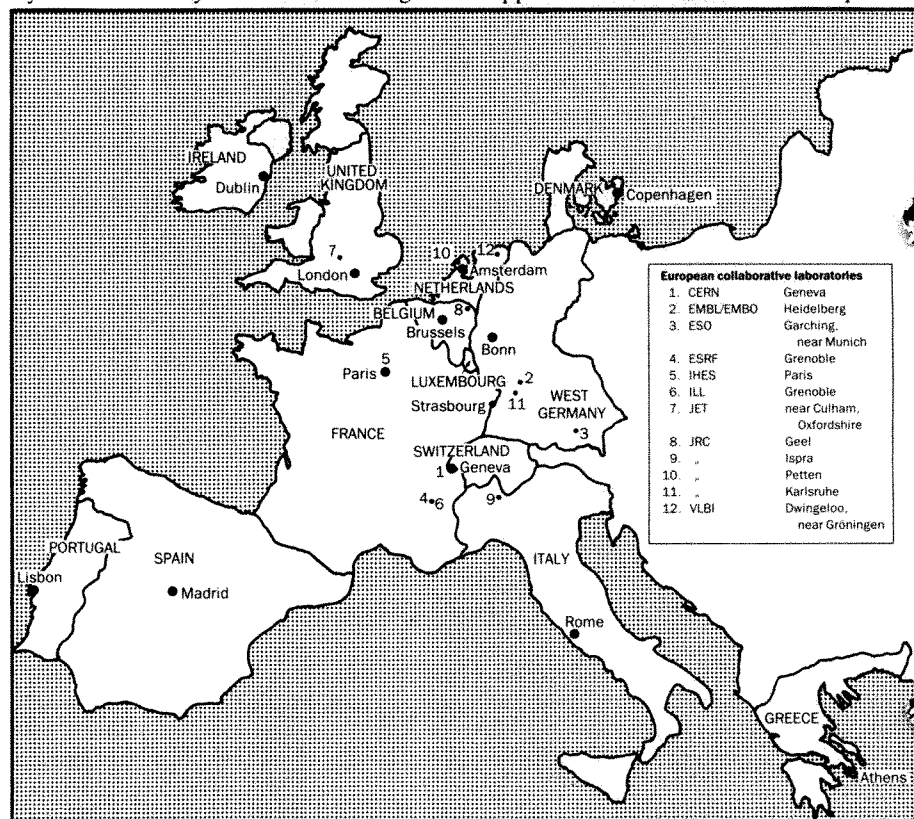
Constitutionally, it is a plain fact that the Treaty of Rome, even as amended by the European Single Act, does not compromise the sovereignty of national governments in the fields of defence, economic (as distinct from trade) policy and foreign affairs. Such forms of collaboration as there may be in these areas are voluntary. Similarly, the Commission's writ does not run to educational policy, either at school or university level, although there is a newly appointed 'task force', run by Welshman Hywel Jones, seeking to

define educational issues to be tackled.

It is also relevant, not merely to the constitutional limits of the Commission's own powers but to its own independence, that commissioners are appointed (and may be removed at the end of their five-year terms) by national governments individually. In office, commissioners function corporately as if they were the cabinet of an administration, but are also given charge of specific portfolios (areas of responsibility); each runs one of the Commission's departments, headed by a director-general, who is in turn responsible for a section of the Commission's staff (most of whom are also seconded to Brussels by member governments).

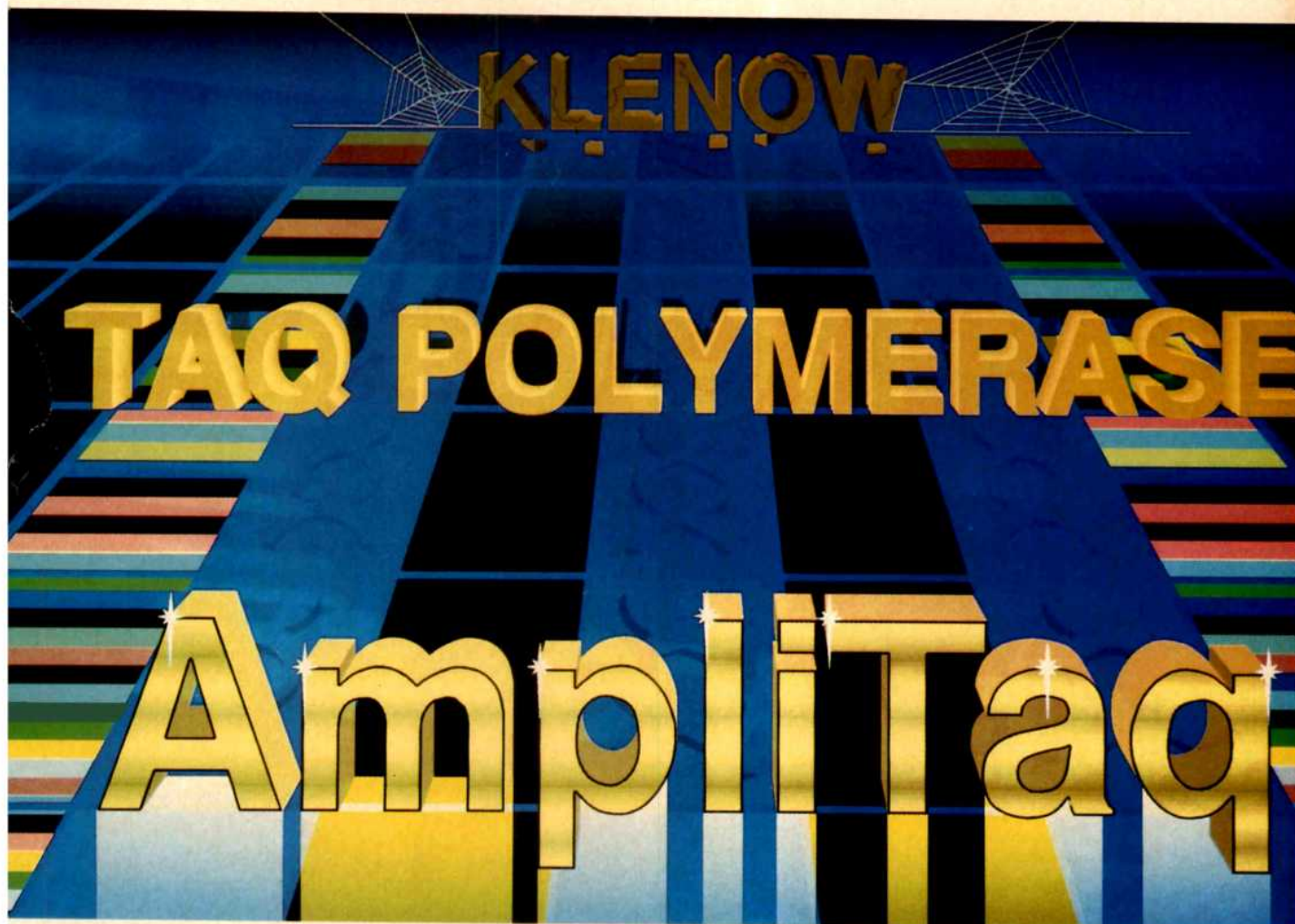
The Commission's independent powers (comparable in many ways to the now-truncated power of the US administration to make war) stem from the provisions of the Treaty of Rome requiring that member states should compete with each other on equal terms. The undoubtedly proper exercise of these powers is nevertheless one of the reasons why the Commission has won a reputation for bumbledom: its directives (on matters such as the definition of particular foodstuffs), which have the force of law in all member states, seem to arouse most derision. Much of the Commission's regulation of environmental standards derives from the same power.

It seems not to have been remarked that, in principle, the Commission's powers to require equal competition would allow it to regulate government support for research and development





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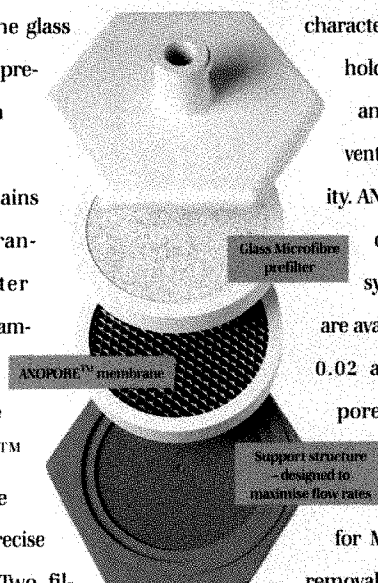
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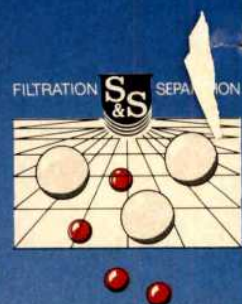
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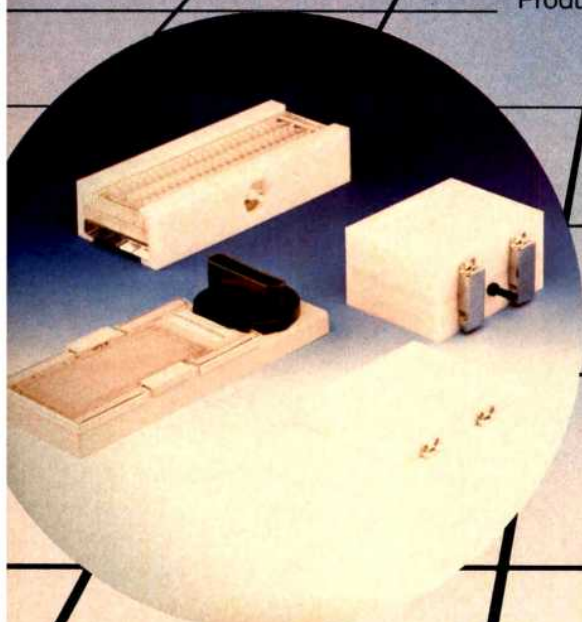


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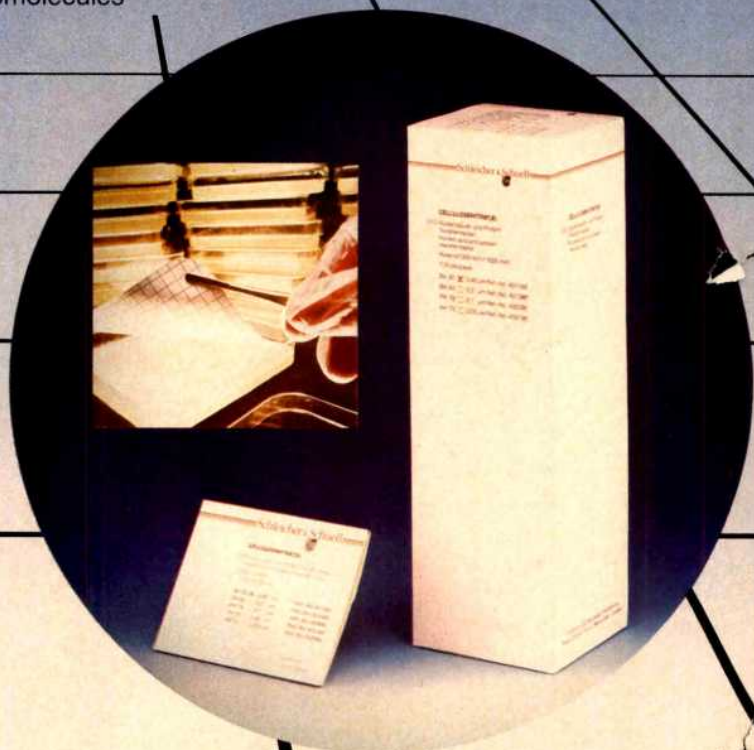
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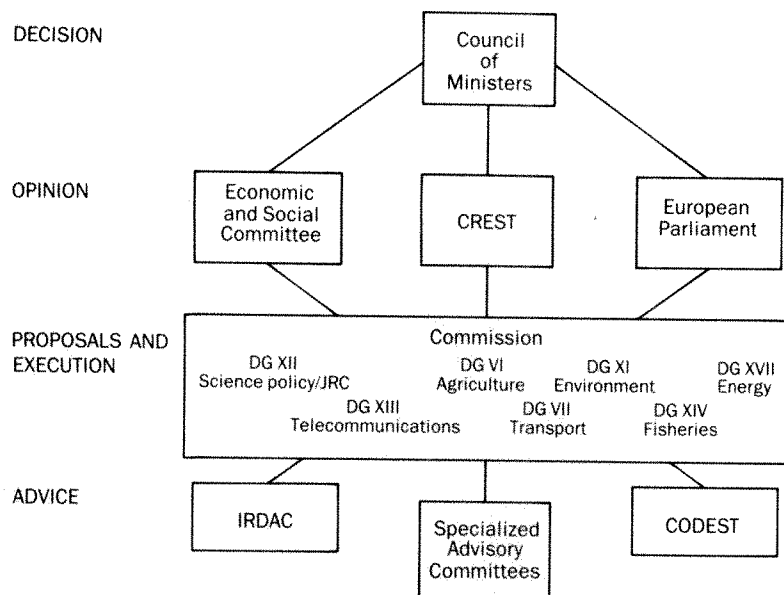
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Commissions in context — the structure of the European bureaucracy.

directed towards the support of its domestic industries.

So far, the Commission appears rather to have concentrated on the development of its own research policy, which is at present a corporate issue spanning at least three of the directorates-general — those responsible for research, industry and environment. Of these, that responsible for research, known as DGXII (pronounced "Dee-Gee-Twelve") plays a crucial role, both as a prime mover behind the five-year 'Framework' programme now half completed, and as the small and only source of support for basic research.

So how fair is the charge of "bureaucrat"? There is no simple answer. But the popular European view that Brussels is filled with a huge army of civil servants bent on interference is wide of the mark. The staff of 17,000 might be held to be small, given the need to administer such a variety of policies in a dozen countries speaking eight acknowledged languages.

The legalism of the Commission's procedures, especially in the drafting of its directives, is probably unavoidable given their force as trans-national legal instruments. The triviality of some of the issues dealt with (the definition of ice-cream, for example) probably reflects the influence of parochial lobbies (to which Brussels is prone), but might be avoided by clear-headed forward planning.

No doubt the directorates-general differ, but first impressions do not suggest that the senior people have a bureaucratic caste of mind. Many of them are Europeans impatient for more rapid progress towards a united Europe. Within a directorate, at least, there is also room for flexibility; DGXII, for example, is frequently able to make small grants of money to help organize meetings on issues that may lead to some collaboration. Nor do the Commission's dealings with the outside world fairly suggest that armies of

pernickety people have deliberately designed forms so complicated as to deter respondents from completing them. On the contrary, the Commission's more common failing is that too much of what it does is done with too much haste, often reflecting people's eagerness to see that money is spent as soon as the appropriate budget has been approved.

Yet there is a sense in which Brussels is a bureaucracy in the worst sense, perhaps unavoidably. The Commission as a whole does not have the flexibility, enjoyed by elected governments, to second-guess

#### IMITATIONS

### Those things that the EEC is not

London

POST-WAR interests in cooperation have littered Europe with international organizations which are often confused with the EEC. Here is a brief guide to them:

- The Council of Europe is a strictly European organization meant to foster collaboration in the fields of law and social policy (which may include, for example, the regulation of research). There are now 26 members. Its chief functions are to negotiate international conventions, which have the force of law in member states; the best-known is the Convention on Human Rights, signed in 1950, accompanied by a European Court of Human Rights which hears complaints by individuals against their governments on the recommendation of the European Commission on Human Rights. The council's headquarters are in Strasbourg.

- The Organization for Economic Cooperation and Development or OECD began life as the Organization for European Economic Cooperation. The name was changed in 1961 with the accession of the United States and Canada and, at a later stage, Japan and Australia. Outside the

their electors, shifting resources in this direction or that as circumstances change. Line-item budgetary control is implicit in everything its political masters allow it to do. Worse, the commission's own procedures are not as coherent as they should be.

For such a young organization as the Commission (or perhaps precisely for that reason), the directorates-general are surprisingly jealous of each other's powers of decision. This is the mechanism by which the Commission appears frequently to reach nonsensical and corrosive decisions on questions such as the use of hormones in growing beef cattle; the power of decision lay with those responsible for agriculture and environment, who chose not to consult those in DGXII who would certainly have known better.

The question whether Brussels might arrange to seem less bureaucratic is a question of its own department. Of necessity, the mostly intelligent and frequently idealistic people working for the Commission differ from those working for national governments in their conviction that the destiny of Europe is to be united. They naturally regard the member governments as entities that must be helped, and sometimes hectored, to share the same vision; with the passage of 30 years, their signature of the Treaty of Rome seems to count for little in Brussels now. Could that be why there seems always to be at least one, and sometimes several, among the member governments which is or are uneasy with the process they began? □

field of economics, OECD has a section of science and technology best-known for its collections of statistics on scientific manpower and spending and for its occasional reviews of national science policies. OECD also operates the Nuclear Energy Agency and the International Energy Agency. The organization is based in Paris.

- The Western European Union is a treaty between seven governments, including Canada and the United States, which among other things is the forum in which the West German government has undertaken never to make nuclear weapons and in which Britain and the United States have undertaken to maintain armed forces in mainland Europe. The headquarters are in London.

- EUREKA is the organization set up on a French initiative in 1985 to arrange for European collaboration on the development of marketable products. Originally advocated as a mechanism for stimulating European industry because, at the time, it was suspected that US industry would be stimulated by the Strategic Defense Initiative (SDI), Eureka now has an administration based in Brussels □



# Budget an endless bone of contention

## Brussels

By the standards of many governments which are members of the EEC, the Commission has a substantial budget for research. In the five-year period ending with 1991, the Commission will have been spending roughly 1,500 million ECU a year, but the research people at Brussels are now excited by the prospect that their collective budget will be increased to 2,500 million ECU a year from about now.

Even at the present level of spending, the Commission's research budget is, in round numbers, virtually on a par with what Britain calls its Science Budget, spent through the research councils on university and in-house basic research. It is also roughly equivalent to the combined federal subventions in West Germany of the Max-Planck Gesellschaft and the Deutsche Forschungsgemeinschaft. But officials here insist that their budget is roughly 4 per cent only of total European spending on research.

The prospect that there will now be an increase seems to be a little-noticed consequence of the budget agreement in the closing days of 1988 at which member governments agreed that there should be a further squeeze on the cost of supporting European agriculture. As yet, there are no firm plans for spending the extra money. (There are a few suggestions elsewhere in this section of *Nature*.)

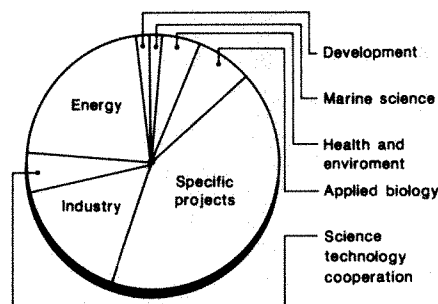
This is a far cry from the second half of 1986, when it seemed as if the British government would carry its opposition even to a reduced programme to extreme lengths. Earlier that year, the British and West German governments had objected both to the scale and the character of what the Commission calls its "framework" programme. Even after the West German position had been modified, the British government persisted with its opposition, acquiescing only after 1988 had begun.

Curiously, direct authority for the Commission's research programme derives only from the Single Act signed in February 1986, which leaves the Commission in no doubt of its responsibilities. The chief task is to strengthen European industry and to help make it more competitive. Roughly 42 per cent of spending in the current programme is under the heading of "Towards a large market", most of it in telecommunications, but 100 million ECU is set aside for traffic and travel projects. Energy (22 per cent), industrial modernization (16 per cent) and applied biology (5 per cent) consume a little more than that (see figure).

The general way of working in the industrial field is that the Commission offers research and development grants on a

50:50 matching basis to consortia of industrial companies or other organizations (such as universities and national government laboratories). A precondition of an award is that the partners must be drawn from at least two European countries. The best-known of these programmes is the telecommunications programme, ESPRIT, on which 2,350 million ECU will have been spent between 1986 and the closing months of 1992.

But the Commission also has research projects which are more particularly its own, outstanding among which is the controlled (and high-temperature) thermonuclear fusion programme whose chief embodiment is the JET (for Joint European Torus) thermonuclear tokamak device in Oxfordshire, England. This pro-



The Commission's current research spending programme will have cost the EEC more than 1,200 million ECU over six years by the time the present phase comes to an end in 1991. (The Commission meets 80 per cent of the total cost.) The design of the next machine, called NET (for Next European Torus) is under way but a decision on its construction (and even siting) has yet to be made. The Commission also contributes to the cost of some national thermonuclear programmes as at Garching (West Germany) and Frascati (Italy).

More contentiously under the Commission's own control are the laboratories grouped together under the name Joint Research Centre (see page 732), mostly inherited from the Euratom organization (which, like the European Iron and Steel Community, was one of the precursors of the EEC). There are four laboratories in Belgium (Geel), Italy (Ispra), the Netherlands (Petten) and West Germany (Karlsruhe), but the work is organized into nine divisions (called institutes), dealing with topics as different as transuranic elements (Karlsruhe), materials (Petten) and environmental safety (Ispra).

The centre's budget problems have their roots in EEC history. Euratom was conceived of as a research organization, but later acquired responsibility for the operation within Europe of safeguards on the use of fissile materials. When Eur-

atom's member states were full of enthusiasm for nuclear energy, it seemed natural that the organization should be ready to build prototype reactors of all kinds, for which purpose research stations were plainly needed. But then the gilt disappeared from that gingerbread.

Ispra, the largest site, has been consistently the most troublesome. Now, after successive painful reorganizations, the laboratory is on the way to being a kind of European Standards Laboratory with responsibilities, on the side, for nuclear reactor safety and environmental safety issues. But the Commission's continued support of the Joint Research Centre was one of the causes of trouble in 1986.

By the standards of these expenditures basic research comes a poor last (page 734). Spending on the relevant part, what is called the EEC's stimulation programme has been fixed at 167 million ECU over the five years to the end of 1992 (up from a total of 60 million ECU in the previous three-year period). Transnational collaboration is a precondition for a successful application, but matching funds are not required.

On the face of things in Brussels, there is general contentment with this pattern of spending. The common goal is to create the single market and somehow to arrange that it is competitive with the rest of the world. If there is a research programme, and especially a substantial one, is it not self-evident that it should be pointed in the same direction?

To outsiders, that logic may not be nearly as compelling. If, for example, the objective of the creation of the single market is that competition between companies should be free and efficient, without reference to their geographical location, will it continue to make sense that most forms of EEC research support should be devised so as to marry efficient companies with less efficient collaborators? And if the Community is concerned about its supply of skilled manpower, might there not be something to be said for strengthening the infrastructure?

Asking such questions in Brussels does not physically endanger one's person; indeed, people are very polite and are willing to acknowledge that they are questions that should be asked. But of whom? The Commission has problems enough on its mind, the European Parliament is preoccupied with the environment and bothered by the discomforts the single market will bring to its constituents. The main council (which meets only twice a year) will have horrendous political disagreements, such as that over monetary union, to resolve by 1992, while the sub-council on research could hardly settle on a change of direction without approval from above. Yet it will be a pity for Europe's dreams to go unanswered because nobody has the time to listen to them. □



## INDUSTRIAL RESEARCH

# Sponsored research but with strings attached

## Brussels

FOR the time being, the cornerstone of the Commission's effort in research is the conviction that European industry must be helped to be more competitive with the outside world, and that the sponsorship of industrial research is the best way of doing that. How well is it doing?

The best-known and the first major collaborative programme is that known as ESPRIT, intended when launched in 1984 as a ten-year programme of research and development in information technology, financed on a cost-sharing basis between the Commission and collaborating companies. (The Commission's share is more than 300 million ECU a year.)

ESPRIT inevitably became the model for later programmes. One of these, BRITE, is a cost-sharing programme aimed at improving the production technology and thus the competitiveness of small and medium-sized enterprises, SMEs in Eurospeak, on which the Commission has spent 175 million ECU on 230 projects since 1985.

The diversity of these programmes is widely remarked upon, as is the enthusiasm of those who take part. ESPRIT, for example, covers everything from the design of the semiconductor elements of computers to the development of software and hardware for desk-top publishing.

Out of the first of these interests has grown the programme called JESSI on the development of submicron computer chips (see *Nature* 337, 682; 23 February 1989). Garcia Aroyo, the DGXII director with responsibility for technological research, appears sanguine at the prospect that his colleagues will soon be labouring under 800-900 applications for funds to support industrial research in production technology and materials development. BRITE was merged with a parallel programme on materials development from the beginning of this year; the closing date for applications under the combined programme (on which the Commission will spend 500 million ECU in the next four years) is 12 May.

The rules are the same. Successful applications must involve industrial companies from more than one European country (and not more than two-thirds of the total grant can finish up in any one country). Companies must be genuinely independent of each other (which means that the collaboration cannot simply be a means by which one company and its subsidiaries elsewhere collaborate on work they might have done anyway). A small proportion of the funds (up to 7 per cent) may be spent on proposals by university consortia for generic investigations provi-

ded that the proposals are endorsed by industrial companies.

Projects must be original and preferably applicable in more than one industry. Interestingly, companies from countries in the European Free Trade Area (Norway, Sweden, Finland, Austria and Switzerland) may take part, but cannot receive Commission funds (and may have to contribute modestly to administrative costs). Research projects must be "pre-competitive". This time round, there is also a scheme for providing up to 75 per cent of the cost of a feasibility study (up to a maximum of 25,000 ECU).

This year's programme has plainly been much influenced by the report of an influential evaluation panel under Yves Farge, vice-president for research and development at the French company Pechiney et Cie., which reported last July. That panel, for example, recommended the merging of the BRITE and advanced materials (EURAM) programmes.

The Commission has also, in a late amendment of its proposals, followed the recommendation that universities collaborating in industrial projects should be recompensed by the Commission for the full cost of their work. (Under the old arrangements, industrial partners would usually pay the other half of the cost.)

Of the questions raised by the Farge committee, those implicitly answered so far are procedural in character. More fundamental issues are unresolved, conspicuously the panel's concern at what it called a "major contradiction" in the Commission's restriction of the programmes it would support to those judged "pre-competitive".

The principle is that the Commission will not support industrial development work leading directly to marketable products or usable processes, but only industrial research of a generic character on which more pointed research programmes can be based. The experience of the BRITE programme apparently showed that some "excellent projects" were not supported because they were judged to be "too competitive", but that the fear of falling into that trap pushed other applicants "upstream", with the consequence that the industrial interest of their projects was diminished. This complaint is also a dilemma for the Commission. Relaxing the pre-competitive rule would place the Commission in the position of providing direct support for commercial development by individual companies.

But the Farge panel points to the recent amendment of anti-trust legislation in the United States allowing research and development partnerships, and says that

## SELF-EVALUATION

## Rooting for grants

SELF-EVALUATION is the price the Commission has to pay for its existence. Councils of ministers insist upon it. But one official describes the process as the administrative equivalent of repeatedly digging up a newly planted tree to see whether its roots are growing.

The Farge panel, for example, was in a position to attempt to estimate the economic benefits that would flow from the two first phases of the BRITE programme by means of a questionnaire to the industrial companies participating.

Among other things, participants were asked to estimate the degree to which the project on which they were working would increase their annual turnover, from which the Farge panel estimated that the total increase of annual production resulting from BRITE projects would exceed 2,000 million ECU. But the panel also noted that the same companies planned to commit only "modest" sums to the further development of the outcomes of their projects, for which reason it suggests that the economic benefits expected of the projects should "be viewed with some caution". □

"the law should be the servant of the Community, not its master". The panel also complains that the Commission, in its management of its research programmes, has not been sufficiently explicit about its objectives.

More generally, the panel pointed out that it had not been able to put the BRITE programme "into perspective in a coherent approach to an industrial research and development policy". This criticism appears only partly to have been met in the new phase of the programme.

BRITE covered nine disparate fields such as computer-aided design (CAD) and production technology for flexible materials (mostly textiles). Its successor has only five broad headings, which may be thought a step towards the definition of a strategy. Three are aeronautics, advanced materials and design, quality and reliability. There are also subprogrammes for the application of known manufacturing technology and the development of novel techniques and processes.

The Farge panel was also concerned that the Commission's own judgement of which projects to support is excessively technical, and insufficiently informed by business considerations. (The Commission's technical staff do get high marks for competence and enthusiasm.) The consequence, the panel said last July, is that there was too much "technology push" and not enough "market pull" in the BRITE programme.

In the nature of things at Brussels, it is too soon to know how the next evaluation panel will rate the achievements of the new programme. Much will depend on how the Commission's officials adapt their choices to the Farge's panel's pleas. The test will be whether Europe's middle-sized businesses are markedly more efficient. □

# Linking an optical cable to every house

## Brussels

WHO knew that Europe is building the hardware and software for a broad-band communications network that will begin to be a reality six years or less from now? The goal is to develop, by 1995, the technical ingredients required for a digital communications network capable of distributing through optical cables both high-definition video signals and huge quantities of data. Brussels seems confident that the project will succeed.

In many ways, the RACE project is what Pierre Aigrain, the French government's chief adviser on science and technology in the late 1960s, was then pleading for (to the EEC). But the new project's ambitions go far beyond Aigrain's hopes. One of the objectives, for example, is that all European dwellings, not simply business offices, should eventually be connected to the network. Meanwhile, there will be high-definition television for all, and pan-European mobile cellular telephony.

According to Roland Hüber, the director in charge of what is called Directorate F (a branch of DGXIII, the Commission's telecommunications ministry), RACE has now let 85 contracts to groups of companies and other organizations (on the Commission's usual 50:50 basis). More than 230 companies are involved, including all the major manufacturers of telecommunications equipment in Europe (together with AT&T and IBM). There are also 89 universities and other independent research organizations, as well as 11 PTTs. Collaborators from the European Free Trade Area are welcome.

As a means of organizing a major tech-

nical project, RACE is itself an innovation (which has been replicated in many of the Commission's projects). The pattern is determined by the circumstance that the Commission is merely the stimulator of innovation, not its eventual executor. Even so, the first step seems to have been to define objectives and to carry out a critical-path analysis for their attainment. But the objectives are not merely technical — the programme supposes RACE must also demonstrate useful applications of broadband networks, which means stimulating demand, and must then study some of the almost sociological problems that are bound to arise so as to demonstrate to eventual investors in the network that their money will be returned.

A further difficulty is that the Commission's funds cannot possibly be stretched to cover all the developments that will be needed for such a project, whence the decision that the Commission should advertise all the tasks that will have to be carried out, but to let contracts for the critical nodes and for a proportion of the others, leaving it to the competitive instincts of the disappointed applicants to pursue projects with their own resources.

The earlier phases of RACE (which began formally at the beginning of 1988) are largely technical, ranging from software development to the investigation of indium phosphide as an alternative to barium niobate in optical switching. Now, while the first crop of projects is under way, the Commission is looking for demonstrations of applications.

Even these phases of the programme offer everybody something. One of the

pilot application projects approved this year, for example, involves 15 European museums (the majority in West Germany) which have undertaken to develop means of displaying museum objects on workstation screens, together with associated sounds and images, so as to make museum collections generally available. The project requires the development of techniques for managing archives by means of expert systems. RACE is interested because similar techniques could be applicable in business. There are also two publishing projects — one involving the rapid transmission and processing of information that may be provided in different forms, the other more specifically concerned with the transmission and local production of newspapers.

A large part of the explanation for change of climate is that the Commission can now boast of having won the collaboration of Europe's traditionally quarrelsome PTTs. One theme running through the report of a strategic audit of the project carried out by a team in which PTT managers were prominent is that national governments must quickly make up their minds about the regulatory framework within which the communications network will operate.

RACE is one of the EEC's more obscure acronyms (standing for R&D in Advanced Communications for Europe), but the word epitomizes the sense of urgency in its conception. The project owes its existence to the recognition in 1985 that the development of broad-band communications systems in Japan and the United States was ahead of that in Europe, and to a calculation that European companies and PTTs, left to their own devices, would not be able to bridge the gap.

By 1986, the Commission was asking for 800 million ECU to support its work-plan, but the request was trimmed to 550 million ECU in the framework budget eventually agreed. Now the Commission is asking that some of the funds should be restored, as it has emerged that many of the projects it has backed will need five, not three, years to complete.

The management of the programme sets great store by the biannual meetings at which all the project teams are represented. Otherwise, projects are autonomous and, as with other industrial research supported by the Commission, members of the project teams make their own arrangements for sharing patent rights and, in due course, such profits as may flow from their development. But the Commission has the right to require that patent rights should be licensed on reasonable terms to other European companies. Beginning in 1992, it is intended that there should be demonstrations of the kinds of integrated networks that may be built from the components that will by then have been developed, at

## APPLIED BIOLOGY

# Travelling a slow road to new technology

## Brussels

THE Commission's research strategy gives applied biology a central place, but progress has been slower than people in Brussels were hoping as long ago as 1980, when the Commission began modestly to support biotechnology projects. Not that enthusiasm has dimmed, but regulatory hesitations by some member states are one cause of delay. Another is that the European blend of well-established pharmaceutical and chemical companies and small start-up companies does not easily lend itself to the kind of financing with which the Commission is most familiar.

The Commission nevertheless maintains a kind of cheerleading organization called the Concertation Unit for Biotechnology in Europe (or CUBE) which is a fertile source of ideas and, occasionally, of smallish pro-

grammes backed by Commission funds. One of its tasks is to steer Europe towards applications of biology that will not further increase present embarrassing overproduction of many kinds of foodstuffs, whence the strategy of encouraging the development of new kinds of crops having a greater nutritional value or containing important industrial raw materials. Using biotechnology in pest control is another goal, as is the development of techniques for using the whole of a crop plant — not just, as in the case of cereals, the seeds.

The name for the new programme, launched last year, is ECLAIR. The budget is a comparatively modest 72 million ECU over five years. But the programme side-steps contentious questions about the release into the field of genetically engineered organisms. □



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DNA researchers.**

first on a laboratory scale, afterwards more generally.

In the end, the success of RACE will hang on the extent to which it influences the development of a broad-band communications network in Europe — and when. Much will depend on the speed and the vigour with which national governments are prepared not merely to enforce common technical standards but to create market conditions in which broad-band communications can prosper.

There are thus political as well as technical problems to solve, not the least of which is that of requiring European PTTs (most of which remain nationalized industries) to allow outside commercial organizations to lease communications channels in the broad-band network for

the provision of what are called value-added services — video signal transmission, for example.

The obvious danger is that different governments may choose to deregulate their PTTs in different ways, thus replacing the present system in which monopolies do what they wish within their territories to one in which national patterns are as different from each other as at present. The Commission spelled out the dangers in a paper published last year, but it has no power to turn its wishes into reality. RACE itself seems convinced that the pace of technical change will shorten its own timetable, but that may be an over-enthusiastic view. Political difficulties that remain unsurmounted could work the other way. □

## RESEARCH FOR INDUSTRY

# Cause and effect in a competitive world

### Brussels

THE European Commission is outspoken about its policy on industrial research, and on research in general. Starting from the calculation that the twelve EEC members spend less on research and development than the United States, both in absolute terms and relative to gross domestic product (GDP), and less than Japan by the second yardstick, the Commission concludes that the objective of its research policy must be to make Europe "competitive", pursuing "where appropriate, its own technological options".

The figures, conveniently collected in the *First Report on the State of Science and Technology in Europe* presented to the European Parliament at the beginning of this year, are chastening.

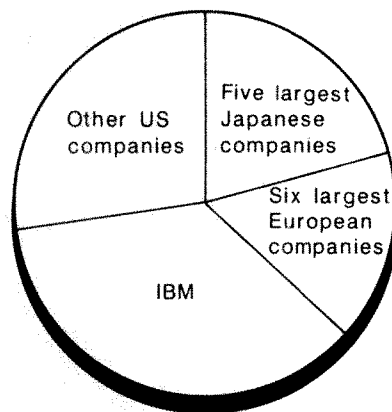
In 1985, EEC spending amounted to 76,250 million ECU, compared with 134,645 million ECU in the United States and 48,056 million in Japan. As a proportion of GDP, European spending was 1.9 per cent, compared with 2.8 per cent in the United States and 2.6 per cent in Japan. The same report notes that non-defence research and development expenditure in Japan is growing twice as quickly (9 per cent a year against 4 per cent a year) as in Europe and the United States.

The Commission is also disturbed by what seems to be a manpower imbalance. According to the US National Science Foundation, in 1986 there were 69 US workers in research and development for every 10,000 in the total labour force, compared with 63.2 per 10,000 in Japan (in 1985) and corresponding figures (in 1984) of 49.1 in West Germany, 41.2 in France and 32.8 in Britain. The report again notes that only the Japanese figure is growing quickly.

The figures for the output end of the

equation are equally sobering. The bulky annexes to the report record in detail that, in industrial sectors as different as chemical production and machine-tool manufacture, EEC performance is in relative decline.

The figure below shows the EEC's estimate of the world's data-processing



market in 1987 secured by the 20 largest companies. The report notes that sales outside Europe of even the largest of the European companies are a small proportion (15 to 20 per cent of their turnover).

The Commission's argument from this point on is simple. There is an imbalance between the EEC and Europe's principal competitors both in efforts in research and development, and in industrial performance. So is it not reasonable to link the two as cause and effect?

The conclusion is that the three goals for EEC research are to improve Europe's international competitiveness, to "increase its capacity to pursue its own scientific and technological options, where necessary by reducing its dependence on others" and "to respond to the needs of society by

improving the quality of life".

The Commission concludes that there are five areas in which European efforts should be concentrated, as follows: information technology and telecommunications, industrial materials, aeronautics "where Europe faces a particularly important competitive challenge", the biological sciences (including biotechnology) and energy.

It is clear that aeronautics will feature prominently in the Commission's next Framework programme. The argument is largely economic, but the competition is exclusively from the United States. The volume of civil air transport is growing at 7 per cent a year, and roughly 40 per cent of operating costs are determined by the cost of the aircraft, while there is also a military market in which, the Commission said in a document published in June last year, US restrictions on the sale of advanced aircraft are worrying.

The plan is that a pilot programme of coordinated research will be drawn up later this year with the intention that there should be a full-blown research programme looking for funds in 1990. For as things are, the Commission said last year, "European industry remains largely divided between national interests. . . . Continuation along this path can only lead to failure".

This important document also deals with the balance between basic and applied research, noting at one point that many European governments in recent years have effected a redistribution of resources from basic to applied research and adding "there is a risk that the pendulum will swing too far . . . just [when] our competitors are placing a greater emphasis on basic research". This is said to be a reference to the growth of the US basic research budget during the Reagan administration and to recent calls in Japan for more attention to the same cause.

On the related issue of the discrepancies within the EEC between the performances of different governments, the report notes that spending on research and development in West Germany, France and Britain (in that order) accounts for 75 per cent of all EEC spending, and that the proportion of GDP spent on research and development ranges from a maximum of 2.8 per cent in West Germany to 1.5 per cent in Italy, 0.8 per cent in Ireland and less than half of that proportion in Greece.

The discrepancy is described even more vividly in an annex to the report prepared by the Irish National Board for Science and Technology, which calculates that the GDP per head of the four poorest countries in the EEC (Ireland, Spain, Portugal and Greece) have an average GDP per head less than 70 per cent of the Community average, but spend (per head) only 25 per cent as much on research and development. □



# A new vision of unity in standards and training

## London

JEAN-PIERRE Contzen, the Belgian director-general of the Commission's Joint Research Centre (JRC), has a vision in which the nine research institutes under his wing will become an amalgam of a standard-setting organization and a means of training Europeans in new technology. Arguing that Europe has too few public research laboratories, his belief that the JRC has a future is infectious. But the future has not always been as certain.

In reality, JRC has only in the past five months emerged from the most serious threat to its existence — the plain discontent of several member states most openly expressed during the long haggle over the present Framework programme. The complaints were that the four laboratories (then distinct) were too costly, too large, badly managed and without function.

The outcome was a review process carried out by a panel under Dr Harry Becker, research director of the Royal Dutch Shell Oil Company, which began in July 1986, and whose chief recommendations were structural and managerial in character. Crucial among these was the decision that the research programme and the administration of JRC should be overseen by an independent board, whose first chairman is Sir John Kendrew, the British Nobel prizewinner.

## Commitology

BRUSSELS is as prolific in the invention of committees as in that of acronyms, and even uses the word *commitology* to describe the theory and practice of committee creation — do committee members represent member states? Who nominates them? Which arm of the EEC reimburses members for travelling expenses? Are decisions by consensus? If a decision is reached by a simple majority, can the chairman break a tie?

This year, each of the nine institutes of the Joint Research Centre (this page) will be blessed with an advisory committee. Most of the Commission's research programmes have one of their own as well.

But in the Commission's administration of science and technology, the most powerful committees are CODEST (page 734), widely respected for its independence; CREST (representing national governments and advising on general strategy), commonly said to be too big to be workable, let alone manageable; and IRDAC (which stands for "Industrial Research and Development Committee"), which suffers from the same faults. □

Contzen, previously DGXII's planner (and thus the architect of the present Framework programme), is in the unusual position of having helped to review the organization he must now administer. Plainly there is some frustration that the legal procedures were completed only in November 1988 (the new plans had to be approved not merely by the Commission and the council, but by the parliament as well). But now, with relief, he says that the new organization is in place.

The most obvious change is verbal: the Commission used to operate four Joint Research Centres, at Karlsruhe (West Germany), Geel (Belgium), Patten (the Netherlands) and Ispra (in the foothills of the Italian Alps), but now there is just the Joint Research Centre, in the singular. The three northern laboratories have been renamed as institutes (but Petten, now called the Institute for Advanced Materials, has a kind of outstation at Ispra); as yet, their research programmes are little changed.

Ispra, always the worry, has seen the most upheaval. Its research programme has been divided into five parts, each of which is renamed as a separate institute. Ispra also becomes the location of the unit intended to provide the rest of the organization with research support, principally in computing.

Part of Contzen's immediate problem is psychological: how to give the 2,180 members of his staff the ideal blend of institutional loyalty and corporate 'solidarity'? The calculation is that the identification of separate institutes dealing with different parts of the research programme is a necessary means of making the separate functions pointed and of making sure that people know what they are about. But there is also a long-term need that people should move more freely than in the past from one programme to another.

Inflexibility is the most obvious difficulty. The Community's procedures are an encumbrance: line-by-line approval and scrutiny of the budget is routine, which means that there is little scope for transferring resources from one project to another as new opportunities arise.

The immobility of the staff is another impediment. Only about 3 per cent move to posts outside JRC each year. What Contzen hopes is that the future pattern of employment may change that. New appointments are now routinely made on a five-year contract, which may be renewed for a second five-year period, but not thereafter; either the person concerned is then appointed for an indefinite period, or he or she must leave.

More than half (53 per cent) are already

employed on short-term contracts such as these. It is now also planned that there should be a system of three-year appointments that will be strictly non-renewable. The first ten posts have been advertised, but Contzen hopes there will be 150 of them in four years. He regards them not merely as a means of recruiting specialists for urgent tasks, but also as a kind of industrial postdoctoral fellowship that may be an innovation in its own right.

JRC also plans to foster its more familiar fellowship schemes (there were 11 graduate students and 24 postdoctoral fellows on the books last year) and to attract more senior people from elsewhere for periods of up to a year. (There were 58 of these sabbatical fellows in 1988.)

Contzen has undertaken that in the next four years, the annual turnover of staff at JRC will increase from 3 per cent to between 5 and 10 per cent a year. A plan to terminate the appointments of 100 staff members will help, but is bound to be a bone of contention in the council of ministers, which has been severely critical of the generosity of previous early retirement schemes.

Salaries at JRC (and at other Commission establishments, such as JET) have always been a contentious issue, partly because they must of necessity be greater than those paid in the EEC's most prosperous member states, partly because they are tax-free. The other side of that coin is that the language and cultural barriers within Europe are still so great that the education of JRC employees' children is likely to be interrupted, as will be their spouses' careers.

The quota system is another stifling influence on JRC. Broadly speaking, the nationality of the professionals on JRC's staff must reflect the size of member states. It is not so much that governments have been anxious to secure for their own nationals posts in a well-paying organization (although there is some of that); others are or have been alarmed that JRC might suck away important parts of their skilled labour force.

The Commission (which is ambivalent on the issue) offers in defence of the system the reflection that it helps to diffuse JRC's work through the Community. But quotas are an irksome impediment to good management; Contzen wishes they would go away. The question of whether there would be quotas (and on what basis) in a united Europe seems not to have been considered.

Despite the impediments, the pattern of research is likely to change more quickly now than in the past. One administrative innovation is the decision that roughly 5 per cent of JRC's resources should be spent on the exploration of new projects.

Under this rubric, in 1988 JRC embarked on investigations of the use of ultrasound (21 kHz) for removing aero-



THE nine new institutes of JRC created by last year's reorganization are as follows:

■ **Institute for Transuranium Elements**, at Karlsruhe (West Germany), may be best known for basic studies of the physical and chemical properties of the actinides, but is also the originator of the project to use ultrasound for removing aerosols.

■ **Central Bureau for Nuclear Measurements**, at Geel (Belgium), has a reputation for precise measurements, most recently of the low energy fission cross-section of  $^{235}\text{U}$ , but is also making precise measurements of the silicon lattice as part of the international redefinition of Avogadro's number.

■ **Institute for Advanced Materials**, at Petten (the Netherlands), is making a transition from the exclusive study of materials under neutron irradiation (for which it was originally established) to the development of new materials and the modification of their properties by surface and other engineering.

■ **Institute for Systems Engineering**, at Ispra (Italy), develops new safeguards techniques for nuclear inspectors, gathers data relating to the safety of thermonuclear fusion reactors but is well-known for a computer program for the assessment of solar input to groups of buildings as part of a general interest in solar heating and conversion.

■ **Institute of Safety Technology**, at Ispra (Italy), is building the reaction wall for the assessment of built structure under mechanical impulsion, but is still concerned with aspects of reactor safety including the study of cooling systems in experimental rigs. It has organized a study of the problems of handling tritium in bulk as will be required in thermonuclear fusion reactors.

■ **Environment Institute**, at Ispra (Italy), traditionally concerned with air and water pollution, has now commissioned a laboratory for the analysis of foodstuffs and drugs.

■ **Institute for Remote Sensing Applications**, at Ispra (Italy), mostly working on the design of novel instruments, has recently proposed to the European Space Agency a scheme for radar sensing of the Earth's surface and plans to use Lidar for the monitoring of fluorine compounds in the atmosphere.

■ **Institute for Prospective Technological Studies**, at Ispra (Italy), is in the process of being organized as a means of contributing to the analysis and formulation of DGXII's policy.

■ **Centre for Information Technologies and Electronics**, at Ispra (Italy), is one name for JRC's computer centre, but also has responsibility for the development of software and will arrange for the design and production of specialized chips. □

sols from large masses of air (which may be useful in dealing with chemical accidents), the applications of neural networks in computing and the further development of a non-destructive monitoring instrument for assessing plutonium in radioactive wastes. High-temperature superconductivity also featured in the programme last year as, no doubt, cold fusion will in 1989.

Broader considerations point to more radical changes in the years ahead. There is, for example, an obvious difficulty at Petten, the chief site of the Institute of Advanced Materials, which also houses JRC's High Flux Reactor originally built as a materials testing tool for Euratom. The reactor is now used for the routine testing of fuel rods of different design as well as for constructional materials used in reactors, but its future is evidently linked to the uncertain future of nuclear power. Accordingly, the operating power and the duty cycle of the reactor have been degraded, if only to prolong its life.

Another strategic consideration is that JRC will be safer if it can work more directly on projects relevant to the Commission's own needs. Data-collection and dissemination (on subjects as different as world shipbuilding and the properties of potentially hazardous chemicals) is an obvious opportunity. So is work on

environmental problems, where Ispra's Institute of the Environment obviously has an advantage.

There is a programme to use satellite observation data (from the French SPOT instruments) to compile agricultural statistics, using five chosen plots of ground in different parts of Europe. Ispra is similarly working on the improvement of instruments for the non-destructive measurement of fissile materials as used by the inspectors who operate nuclear safeguards procedures. (The European Community has secured an undertaking from the International Atomic Energy Agency that its member states' compliance with the Nuclear Non-Proliferation Treaty will be assured by the reports of its own inspectors.)

In the same spirit, the research group at Ispra originally concerned with the safety of nuclear reactors, and now renamed the Institute for Safety Technology, has spread its wings also to cover the safety of civil engineering structures and of industrial plants. The group should have finished next month the design of a structure (called a reaction wall) for the measurement of seismic and other mechanical impulses to structures. It has also become a source of expertise in the application of finite-element techniques in the design of engineering structures.

But much else of JRC's work is of a general character, relevant to the Commission's needs only in the most general sense. Conversely, there are fields in which the Commission would not at present turn to JRC for advice on the solution of all its problems — the use of hormones in growing beef cattle, for example, despite Ispra's interest in NMR techniques in the analysis of foodstuffs (the alcoholic content of wine in particular; see the News and Views article on page 708 of this issue) or the development of technical standards in telecommunications (where other organizations are more expert).

Yet the new research commissioner, Filippo Maria Pandolfi, has been saying since he took office at the beginning of this year that he wants DGXII to concentrate more of its resources on 'pre-normative research', which is eurospeak for work that may help to strengthen the Commission's administrative hand, from the standardization of equipment (as in telecommunications) to the basis for environmental standards and even the promulgation of standards used in trade and commerce, the work of old-fashioned standards laboratories for example.

In a broad sense, that is also a central objective for Contzen — but nobody wants JRC to become a kind of International Bureau of Standards for Europe.

Budget pressures will also shape the future pattern of JRC. The Commission has decreed and the board has agreed that JRC should find a greater proportion of its income from outside the Commission's budget, and indeed the annual report for 1988 (issued earlier this month) says that contracts already signed will contribute about 30 per cent to JRC's costs during the four years ending in 1991. But that calculation is something of a fudge.

The external contributions (over four years) include 55 million ECU for the operation of the Petten reactor and 59 million ECU for the support of Commission activity in agriculture, the environment and the like. Genuine external contracts now signed will bring in only just under 20 million ECU by 1991, although JRC's reorganization (and the recognition that its future depends on selling its services) will no doubt increase that amount substantially. But operating costs are less than half the total: 'personnel credits' amount to 132 million ECU out of this year's total budget of 239.4 million ECU.

Indeed, Contzen is also quick to argue that these calculations do not give JRC credit for the work it does as a partner in several of the consortia contracted to work for the Commission's industrial development programme. (The rules prevent programmes such as RACE from paying money to JRC.) Institutes collaborate out of interest, but there is a possibility that royalty income will eventually accrue. □

# Larger budget still too small for comfort

## Brussels

THE Commission's programme for the support of basic research has a new budget (33 million ECU a year until the end of 1991) and also a new name — SCIENCE — which is a triumph for whichever directorate-general looks after the invention of Community acronyms. But the budget, some 3 per cent of the Framework programme, is too small for comfort — and in 1988 the SCIENCE programme made 114 grants worth a total of 36.8 million of ECU.

Not that those who administer the programme are ungrateful for last year's increase, which has virtually doubled the funds available each year, and which represents a very substantial increase over the 6 million ECU available in 1984, in the first year of what was then called the Stimulation Programme. (No less than 1.5 million ECU was then spent on a project in which half a dozen universities planned to collaborate on optical computing.)

The SCIENCE programme is the nearest thing to a grant-making research foundation that there is within the Commission's organization. Its chief objective is to encourage collaboration between basic research groups, almost always by means of grants that supplement the funds available from national sources. The same budget has been used on three recent occasions to make grants to the European Science Foundation to support networks of European researchers.

Applicants for funds should be wary. SCIENCE does not pay for expensive pieces of equipment, nor does it provide running costs while, like all grant-making organizations with small budgets, it is torn between the wish that the projects that it backs will prosper and the fear that, if they do, they will wish to turn themselves into permanent pensioners. Even so, the programme is ready with examples of projects which have gone well — sometimes, well enough to be partners in larger Commission projects under other programmes, the BRITE programme for example.

One of these is the research programme on new permanent magnets typified by the NdFeB alloys which was begun by the Stimulation Programme in 1985 and which now embraces 120 people from 58 different laboratories (most of them, interestingly, at universities). The collaboration is following up the discovery in 1963, by the Sumitomo Special Metals Company and General Motors, that sintered materials with the approximate composition  $\text{Nd}_2\text{Fe}_{14}\text{B}_7$  make permanent magnets of high field strength and coercivity.

The project has the virtue of being interdisciplinary as well as international

— there have been problems of growing single crystals, the determination of their crystal structure (which appears to be layered much as are the the high- $T_c$  superconductors), unravelling the relationship between magnetic properties and microcrystalline structure and fabrication techniques. (One remarkable development is exploitation on an industrial scale of the laboratory curiosity, called decrepitation, in which gaseous hydrogen interacts with solid  $\text{Nd}_2\text{Fe}_{14}\text{B}_7$  newly cast from the melt to reconvert it into a powder.)

The collaboration's biggest prize so far seems to have been the discovery that the partial substitution of Nd by the rare earth element dysprosium (Dy) both increases the coercivity of the alloys and the temperature (below the Curie temperature) at which the exceptional magnetic properties are retained — important because of the temperatures likely to be encountered in the electrical machines incorporating these materials. The project has now won the accolade of a Euroacronym all of its own (CEAM) and support from the programme on advanced materials.

Last year, with more money to spend, CODEST appears to have backed as diverse a range of projects as anybody could wish. The handful of mathematics

SCIENCE PARLIAMENT

## Voice for research in Brussels

### Brussels

DGXII, which unlike most of the other directorates-general at Brussels is not much molested by lobbyists, has a scheme to mend its sense of isolation from its true constituents, the research community. Almost subversively, but keeping well within the rules, it plans to establish an assembly of working scientists which will represent the research community in Brussels.

The scheme is entirely within the gift of the Commission, which is free to decide how to spend the resources set aside for meetings of people. About now, the Commission should have taken the plunge. But some member governments are uneasy, suspecting that DGXII is looking for a means of recruiting scientific advice that will be an alternative to that provided by its own placemen on the innumerable Brussels committees.

The uneasiness may be well placed. The European Assembly in Science and Technology will consist of 200 people, a third of whom will be nominated by each of CODEST (see above), the Commission's basic research committee, the European Science Foundation (ESF, see page 723)

and computer science projects, for example, includes a three-year collaboration between the universities of Aberdeen and Paris-Sud to develop software that, starting from an incomplete theory and a mass of empirical information, will seek more complete laws and define the exceptions to them.

Last year's biggest projects include a 15-laboratory collaboration to make and characterize the structure of single crystals of high- $T_c$  superconductors, which will cost the SCIENCE programme 1.36 million ECU over two years. Another project (involving laboratories in Belgium and the Netherlands with two in France) aims to build a laser capable of generating light intensities of up to  $10^{20} \text{ W cm}^{-2}$  in 30 femto-second pulses, with the objective of following the multiphoton ionization of atoms produced (2.06 million ECU over 3 years).

Nineteen laboratories (nearly half of them British) are planning to collaborate on a basic study of the properties of magnetic recording materials with the deliberate intention of providing support for European industry (1.88 million ECU over 3 years), while the Netherlands Cancer Research Institute, the Pasteur Institute in Paris and the British Medical Research Council's Radiobiology Unit at Chilton, Oxfordshire, plan to pool their expertise in the genetics of the mouse to make a start on mapping the mouse genome (1.27 million ECU over 3 years).

Last year's largest collaboration, in

and national governments (*pro rata* by some rule).

The routine function of the assembly will be that of a panel of referees to whom all kinds of questions may be referred. But DGXII seems genuinely to be looking for new ideas. No doubt there will be opportunities for groups within the assembly, form committees to advocate what seem promising courses of action. There are also plans that the members of the assembly should meet together occasionally. Dreams that the assembly may be the world's first parliament of science are not far beneath the surface.

The practical difficulty may be whether 200 people can adequately represent European science and technology. The nominations not yet made will evidently be crucial. DGXII seems anxious that its assembly should not be mistaken for the European Academy, the feasibility of whose formation is being explored by Sir Arnold Burgen, a past foreign secretary of the Royal Society of London, and with ESF itself, a consortium of national grant-making organizations which has nevertheless sometimes mistaken itself for a European academy. □

## FORWARD LOOK

## Where to go next?

## Brussels

ONE key figure in shaping the future pattern of the Commission's support of research and development is Dr Paolo Fasella, the Italian who has been director-general of DGXII for long enough to command the respect of a substantial part of European science. He is emphatically not a bureaucrat, but an enthusiast.

Last month, Fasella was basking in the outcome of the council meeting on 14 March. The decisions taken then bring to 98 per cent the proportion of the current Framework programme budget which has been approved (but some projects still have to work their way through the parliament). There is now a sense of expectation as people ask themselves what will follow. Will the expected increase of the budget from 1,500 million to 2,500 million ECU by the end of 1991 materialize?

The same meeting of the research council approved one of Fasella's favourite projects — that of using Community funds (30 million ECU by the end of 1991) to make better use of major installations scattered through the Community. The starting-point is the calculation that pieces of equipment as different as radiotelescopes and synchrotron light sources may be under-used because those for whom they were built do not, or no longer, use them fully. A synchrotron, for example, may have been equipped with fewer outlets on its circumference than can be accommodated.

Under the new scheme, operators of large EEC installations may be given grants with which to upgrade them, on condition that the equipment is then made available to researchers elsewhere in the EEC or, at first, elsewhere in Europe. Support will be provided for periods of three to five years. DGXII's hope is that it will soon have some 30 installations on its books.

On the more distant future, Fasella believes that the programmes of industrial research support will continue, even multiply, but that the Commission will find itself paying more attention to the needs of basic research. He says that many of DGXII's research activities, not simply its occasional grants to the European Science Foundation, help to form durable networks of people, which could usefully be multiplied.

H. J. Allgeier, director of the division responsible for planning and evaluation in DGXII and a strong advocate of the nascent aeronautics programme, goes further, acknowledging that it may not be long before the Commission has to face up to the responsibility of "standing behind" research institutions such as CERN and the European Space Agency (ESA). But at this stage, nobody seems willing to go to

the stake for turning the Commission's research programme into EEC's public grant-making agency.

It is not easy to see how this question will get the hearing within EEC that its importance demands. Most member governments are worried about their own economic performance in relation to that of other member states and also share the general view that competing with the United States or Japan (or both) is what EEC is for. They take the simple view that backing industrial research makes sense, but at the same time value the ability (or illusion) of seeming to shape national policy which national grant-making agencies can provide.

It is also the case that the Commission is not constitutionally well-suited to the role of a grant-making agency dedicated to the support of basic research and the pursuit of excellence. There would be obvious political difficulties if the research grants awarded by the Commission's hypothetical grant-making agency were not *pro rata* with member states' estimates of their national importance (by the yardstick of population, GDP or whatever). More seriously, most well-judged research grants would be discriminatory, not involving transnational collaboration, which the Commission is meant to foster.

There are nevertheless two directions in which the Commission could usefully feel out the ground ahead. First, it is anomalous that the Commission has so little direct concern with skill training and higher education; direct support of basic research might be a useful entrée.

Second, it would be entirely consistent with the Commission's present function that it should play a direct part in supporting collaborative research institutions, CERN, ESA and the smaller fry. Member governments would suspect that to be a way of spending their money, by proxy and with less attention than they would exercise themselves, but there are many formulae by which the obvious dangers could be avoided. Why not make a few experiments? The VLBI proposal (page 720) would be a natural starting-point.

Commission support for basic research in member states might also be held germane to the Commission's function if it were regarded as one of the means by which economic imbalances within EEC might be in the long run corrected. There is already a Social Fund providing palliative cash. Why not research funds as well? Given that less prosperous member states' ability and readiness to spend money on basic research will always be deficient, the Commission's intervention may be the only sure way of increasing the general level of research spending in the Community.

which more than 100 researchers from eight Community countries are involved, is the study of plankton in the Antarctic seas organized by the Alfred Wegener Institute at Bremerhaven, in West Germany. Material was collected from the research vessel *Polarstern* during the Antarctic summer just completed, but its analysis will take a further two years (to which the Commission will contribute 551,000 ECU). And six marine science laboratories plan to study Quaternary sedimentation in the North Sea by means of a series of boreholes and with the help of a 1.51 million ECU grant over two years.

Among the grants CODEST did not make last year is that to which *Nature* has given some publicity — the proposal by the European VLBI consortium of radio-astronomers that the Commission should finance its plan for the re-equipment of the collaborating observatories and also support the proposed centre for the analysis of VLBI data at a cost of 17.8 million ECU.

In reality, the VLBI proposal would have consumed the whole of the SCIENCE budget for a year. The issue that remains to be decided is not whether the SCIENCE programme must be enlarged, but whether the Commission should devise a new mechanism for meeting such requests, which is subsidiary to the question of what the Commission's role in basic research is judged to be.

With all that said, in its small way, SCIENCE seems to enjoy a degree of flexibility not common in the Commission's works. Being small no doubt helps, but so does the advisory committee called CODEST which ultimately decides which projects shall succeed. (First, there is a refereeing procedure.) As if in recompense for its time and trouble in cutting up the small cake, CODEST has the right to be consulted about all proposals for developments in science and technology making their way to the Commission.

The chairman of CODEST since 1987 is Sir Peter Swinnerton-Dyer, chairman of the British University Grants Committee until that was transmogrified into the Universities Funding Council, of which he is now the chief executive. Unlike most of the Commission's advisory committees, CODEST members are not representatives of their governments but independent persons, which also gives the committee more legitimacy than that attaching to groups of government nominees.

Given the influence of CODEST in the Commission's scheme of things, as well as its independence, it is probably the institution in the EEC best placed to raise the important question of what the balance between basic and applied research should be. There is not much time left for tackling the question: the next Framework programme will be upon us before we know.



# Unprepared for the single market

Will it be different after 1992? The general expectation that everything in Europe will change when the EEC (at last) becomes a common market is belied by the inadequacy of the preparation for that event.

THE EEC's research programme, the subject of the last part of this survey of European science, has always been controversial and will long remain so. The Treaty of Rome omitted to give the European Commission the power to sponsor research, because in the simple customs union then foreseen, the Commission needed merely the power to create the level playing-field on which companies in member states could compete on equal terms. In the 1950s, before research became a self-conscious component of public policy and long before governments recognized the link between research and prosperity, research belonged with cultural policy squarely within the competence of member states. Both the Iron and Steel Community and Euratom (which predated EEC) had research remits, but they had technical needs to satisfy.

Since then, the Commission has stumbled into research by historical accident — the coincidence of its administrative takeover of the two subsidiary communities with the rise of European anxieties in the 1970s about the 'technology gap' between Europe and the rest of the industrialized world. The Commission has responded manfully, but not always wisely, to this challenge. Its programmes described in the preceding pages are merely the most conspicuous. Yet now, with the prospect of a single market going beyond a mere customs union, there can be no doubt that the Commission has a proper role in research. The Single Act that promises great things for 1992 acknowledges as much. The danger now is that what it does will be the extrapolation into the future of present historical accidents. Several questions arise.

First, there is the matter of administration. In part, the Commission has been unfairly pilloried. The Joint Research Centre (once named in the plural) was in a bad way when the Commission took it over. Now, at least, there is a workable structure and a plan that may succeed (page 732). It should be given a fair chance. The more expensive cost-sharing programmes (page 729) are a more mixed bag. Much of the work they support is valuable — the evaluation panels say so — but they would be more valuable if they were not so often hastily thrown together. Often the Commission will spend well over a year winning approval for some scheme, give client companies and academics a few months in which to apply — and then spend more than a year making legal arrangements for the funds to be released.

Second, there is the matter of what the Commission's research is for. As stated, the purpose is to enhance industrial "competitiveness" while supporting only "precompetitive" industrial research. The Farge panel last year spotted that contradiction. Another, certain to seem sharper after 1992, is whether it will make sense to

insist that industrial research projects qualifying for assistance should always entail transnational collaboration when European competitiveness (with the outside world) could be based on a single efficient company. A further difficulty is that these projects seem to concentrate on innovation (often the 'me-too' kind) rather than efficiency, suggesting that self-sufficiency is the real goal.

Third, there is the matter of the balance between basic and applied research. The Commission has so far concentrated on the second, but acknowledges that Europe's future hangs not only on a torrent of innovation but on a sufficient supply of innovative people. The logic of that is that it should be spending a greater proportion of its funds on basic research, which is the only proven way of systematically turning talented people into innovators. The SCIENCE programme (page 734) is fine, but should be increased and also supplemented by a means of backing long-term institutional endeavours. The planned, but frustrated, VLBI scheme (page 719) is a test-case.

Fourth, there is the matter of the Commission's own dependence on science. Even among its constituents, the Commission has a bad name for making technical decisions in apparent ignorance of the ins and outs of technical questions. The decision last year to ban the use of anabolic steroids in growing beef followed the over-stringent standards for radioactive materials in food adopted in the aftermath of Chernobyl two years ago. But the Commission corporately is not nearly as ignorant as it makes itself seem. It is merely that when technicalities contradict what seem to be political, social or economic imperatives, it is easier to make a decision by neglecting to ask for the inconvenient advice. That may keep member-governments happy, but it is not a way to govern.

Finally, there is the related matter of the Commission's own personnel, researchers on its payroll, for example. As things are, the Commission is compelled by its member governments to operate a quota system: governments do not want to stuff ECUs into other nationals' pockets, especially in such large amounts. Will petty chauvinism of that kind make sense after 1992? And if not, how else are talented people to be employed? The question is again administrative, but it also goes deeper: the Commission expects 1992 to be a milestone on the way to a United States of Europe. If it had its time again, the Commission would no doubt start from somewhere other than the Treaty of Rome, perhaps from a common understanding on external relations. But history does not repeat itself for the benefit of those whom it inconveniences. The Commission's flair for dealing with its officials as if they were European could be another acid test of the validity of its ambitions for Europe. □



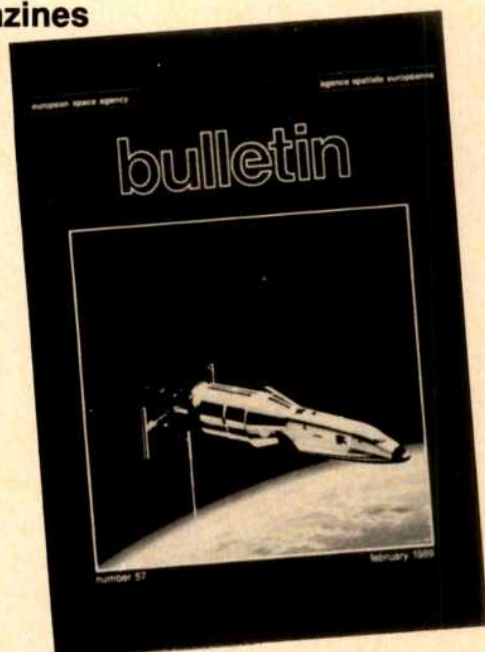
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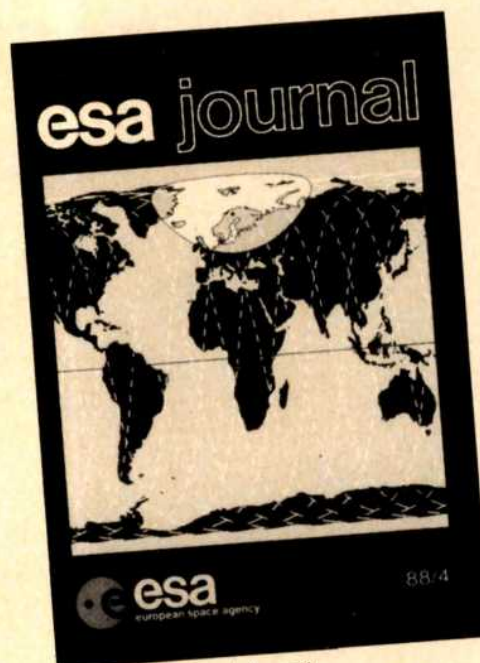
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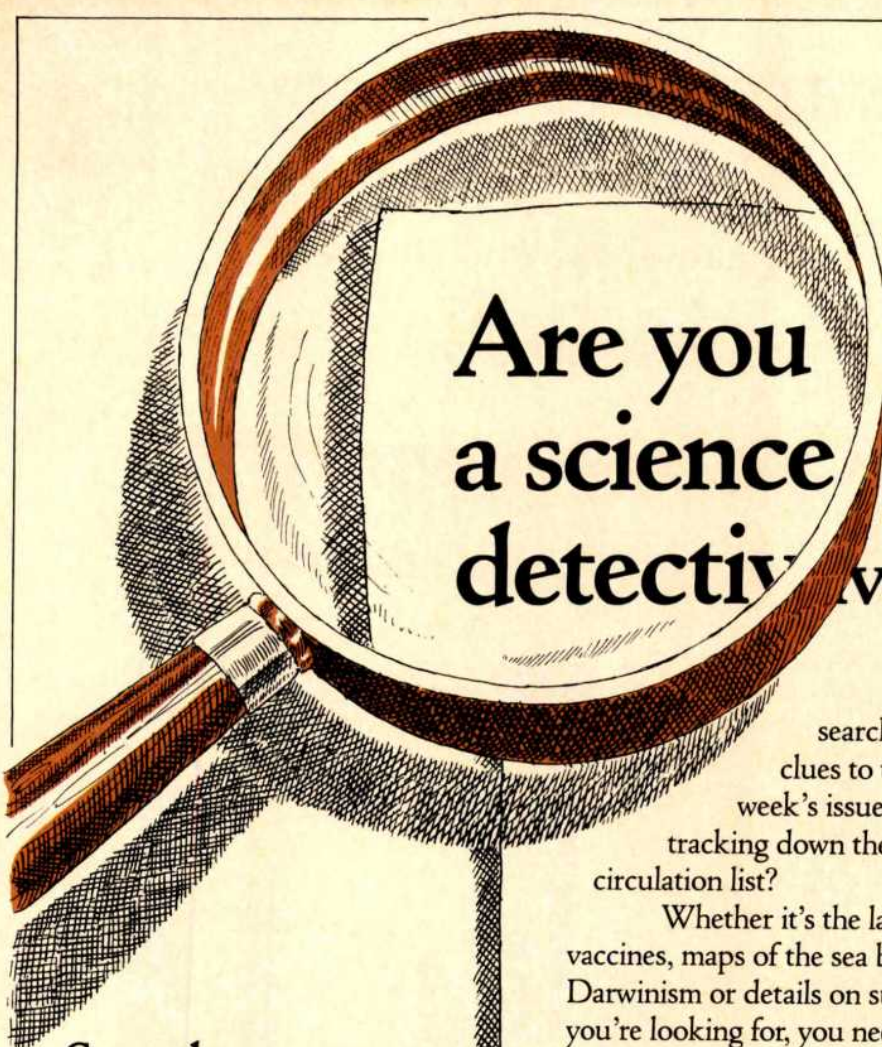
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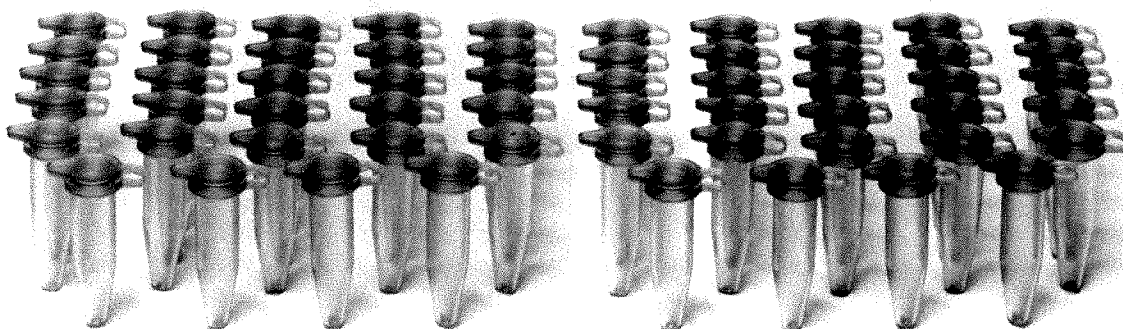
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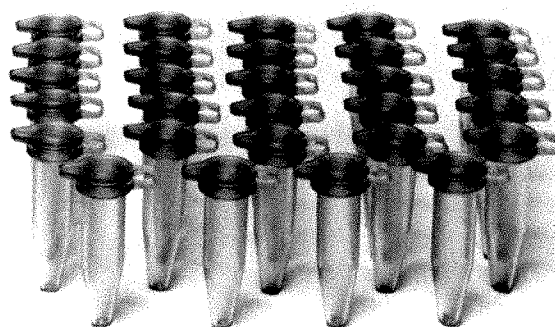
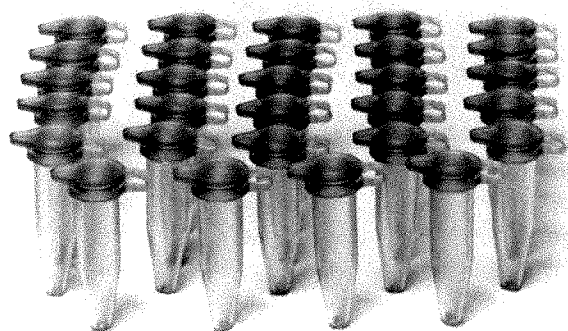
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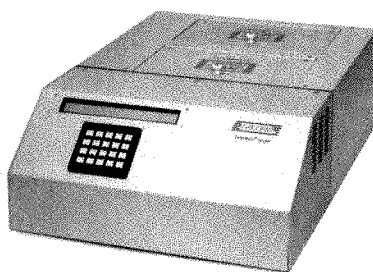
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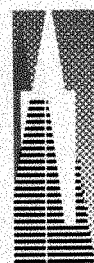
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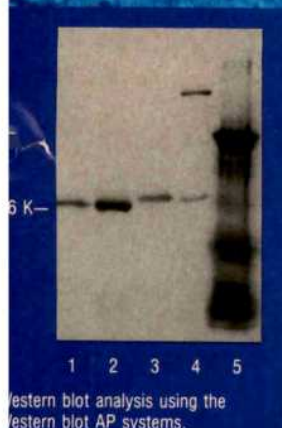
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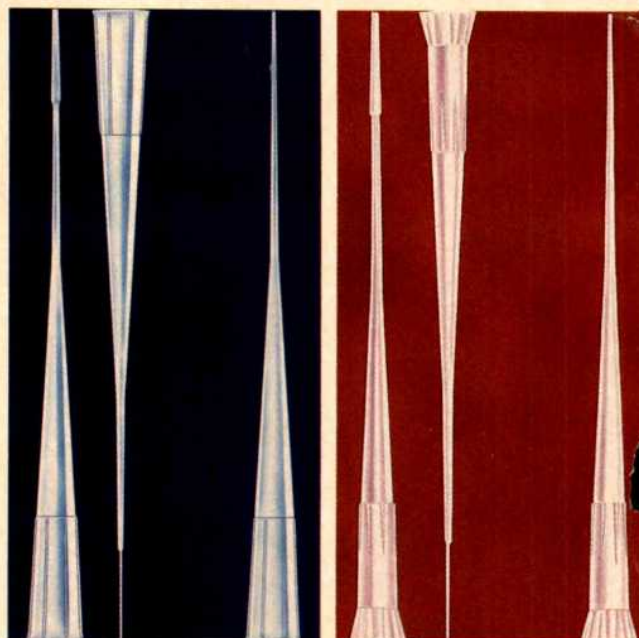
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# Observation of cold nuclear fusion in condensed matter

S. E. Jones\*, E. P. Palmer\*, J. B. Czirr\*, D. L. Decker\*, G. L. Jensen\*, J. M. Thorne\*, S. F. Taylor\* & J. Rafelski†

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When a current is passed through palladium or titanium electrodes immersed in an electrolyte of deuterated water and various metal salts, a small but significant flux of neutrons is detected. Fusion of deuterons within the metal lattice may be the explanation.

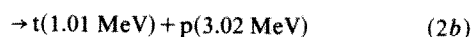
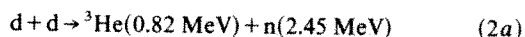
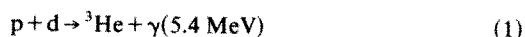
FUSION of the nuclei of isotopes of hydrogen is the principal means of energy production in the high-temperature interiors of stars. In relatively cold terrestrial conditions, the nuclei are surrounded by electrons and can approach one another no more closely than is allowed by the molecular Coulomb barrier. The rate of nuclear fusion in molecular hydrogen is then governed by quantum-mechanical tunnelling through that barrier, or equivalently, the probability of finding the two nuclei at zero separation. In a deuterium molecule, where the equilibrium separation between deuterons (d) is 0.74 Å, the d-d fusion rate is exceedingly slow, about  $10^{-74}$  per  $D_2$  molecule per second<sup>1</sup>.

By replacing the electron in a hydrogen molecular ion with a more massive charged particle, the fusion rate is greatly increased. In muon-catalysed fusion, the internuclear separation is reduced by a factor of  $\sim 200$  (the ratio of the muon to electron mass), and the nuclear fusion rate correspondingly increases by about eighty orders of magnitude. Muon-catalysed fusion has been shown to be an effective means of rapidly inducing fusion reactions in low-temperature mixtures of hydrogen isotopes<sup>2,3</sup>.

A hypothetical quasi-particle a few times as massive as the electron would increase the cold fusion rate to readily measurable levels of  $\sim 10^{-20}$  fusions per d-d molecule per second<sup>1</sup>. The results reported here imply that a comparable distortion of the internuclear wavefunction can be realized when hydrogen isotope nuclei are loaded into metals under certain conditions. We have discovered a means of inducing nuclear fusion without the use of either high temperatures or radioactive muons.

## Indirect evidence

Observations of naturally occurring  $^3\text{He}$  in the Earth suggested to us new directions for laboratory investigations of nuclear fusion in condensed matter.  $^3\text{He}$  is produced by the following fusion reactions:



Tritium (t) decays with a 12.4-yr half-life to produce  $^3\text{He}$ . The well established high  $^3\text{He}/^4\text{He}$  ratio in solids, liquids and gases associated with volcanoes and other areas of high heat flow<sup>4-6</sup> suggests fusion as a possible source for the  $^3\text{He}$ .

To estimate a possible rate of fusion in the Earth, we assume a simple, steady-state model in which the known flux of  $^3\text{He}$  out of the mantle,  $2 \times 10^{19}$   $^3\text{He}$  atoms per second<sup>7</sup>, arises from p-d fusion occurring uniformly in the mantle water reservoir, taken as  $\sim 1.4 \times 10^{24}$  g (R. Poreda, personal communication). Note that if the Earth contains 'primordial'  $^3\text{He}$ , our calculated

rate will be an upper limit; on the other hand, if fusion-produced  $^3\text{He}$  is stored in the mantle (so that the outward flux does not equal the production rate), our value will be a lower limit. As each p-d fusion produces one  $^3\text{He}$  atom, and as the isotopic abundance of deuterium in water is  $\sim 1.5 \times 10^{-4}$  deuterons per proton, we infer a geological fusion rate constant,  $\lambda_f$ , of

$$\lambda_f \approx \frac{2 \times 10^{19} \text{ } ^3\text{He atoms s}^{-1}}{1.4 \times 10^{43} \text{ deuterons}} \quad (3)$$

$$\approx 10^{-24} \text{ fusions d}^{-1} \text{ s}^{-1}$$

This rate is fifty orders of magnitude larger than that expected in an isolated HD molecule, and fusion at this rate could be detected if reproduced in the laboratory.

Cold nuclear fusion may be important in celestial bodies other than the Earth. Jupiter, for example, radiates about twice as much heat as it receives from the Sun. It is interesting to consider whether cold nuclear fusion in the core of Jupiter, which is probably metallic hydrogen plus iron silicate, could account for its excess heat. Heat is radiated at an approximate rate of  $10^{18}$  watts, which could be produced by p-d fusions occurring at a rate of  $10^{30} \text{ s}^{-1}$ . Assuming a core of radius  $4.6 \times 10^9$  cm, containing mostly hydrogen, with density  $\sim 10 \text{ g cm}^{-3}$  and a deuteron/proton ratio of  $\sim 10^{-4}$ , we deduce a required p-d fusion rate of  $\lambda_f \approx 10^{-19}$  fusions  $\text{d}^{-1} \text{ s}^{-1}$  if all the heat derives from fusion. Catalysed nuclear fusion at this rate could be readily measured in the laboratory.

Further evidence for cold nuclear fusion in condensed matter comes from studies of  $^3\text{He}$  and  $^4\text{He}$  in metals. There have been several reports of high  $^3\text{He}$  concentrations in metal crucibles and foils (H. Craig, R. Poreda, A. Nier, personal communications), consistent with *in situ* formation by cold fusion. In particular, Mamyrin *et al.*<sup>8</sup> report the occurrence of patchy, high concentrations of  $^3\text{He}$  in a number of metal foils. Electrolytic refining of the metals could have provided the appropriate conditions for the cold nuclear fusion reactions (1) and possibly (2). Among several possible explanations for the observations, the authors suggest an analogue of muon catalysis<sup>8</sup>.

## Detection of cold-fusion neutrons

The considerations outlined above led to laboratory experiments performed at Brigham Young University to determine whether cold nuclear fusion can actually occur in condensed matter. We now report the observation of deuteron-deuteron fusion at room temperature during low-voltage electrolytic infusion of deuterons into metallic titanium or palladium electrodes. The fusion reaction (2a) is apparently catalysed by the deposition of  $d^+$  and metal ions from the electrolyte at (and into) the negative electrode. Neutrons with an energy of  $\sim 2.5$  MeV are clearly detected with a sensitive neutron spectrometer. The experimental layout is shown in Fig. 1.

The neutron spectrometer, developed at Brigham Young University over the past few years (ref. 9 and manuscript in preparation) has been crucial to the identification of this cold fusion process. The detector consists of a liquid organic scintillator (BC-505) contained in a glass cylinder 12.5 cm in diameter, in



which three glass scintillator plates doped with lithium-6 are embedded. Neutrons deposit energy in the liquid scintillator through multiple collisions, and the resulting light output yields energy information. As their energy decreases, the neutrons are scavenged by  $^6\text{Li}$  nuclei, and the reaction  $n + ^6\text{Li} \rightarrow t + ^4\text{He}$  results in scintillations in the glass. Pulse shapes and amplitudes from the two scintillators differ; the two distinct signals are registered by two photomultiplier tubes, whose signals are summed. A coincidence of identified signals from the two media within  $20\ \mu\text{s}$  identifies an incoming neutron that has stopped in the detector.

The spectrometer was calibrated using 2.9- and 5.2-MeV neutrons generated by deuteron-deuteron interactions at  $90^\circ$  and  $0^\circ$ , respectively, with respect to a deuteron beam from a Van de Graaff accelerator. The observed energy spectra show broad structures which imply that 2.45-MeV neutrons should appear in the multichannel analyser spectrum in channels 45–150. The stability of the detector system was checked between data runs by measuring the counting rate for fission neutrons from a broad-spectrum californium-252 source.

We have performed extensive tests to verify that the neutron spectrometer does not respond preferentially in this pulse height range to other sources of radiation such as thermal neutrons. In particular, we made unsuccessful efforts to generate false 2.5-MeV neutron 'signals' by using various  $\gamma$ -ray and neutron sources and by turning auxiliary equipment on and off. Neutron-producing machines such as the Van de Graaff accelerators were off during all foreground and background runs.

Many background runs were made using operating cells (described below) containing standard electrodes and electrolytes, except that  $\text{H}_2\text{O}$  replaced the  $\text{D}_2\text{O}$ ; other background runs were made using both new and previously used standard cells containing  $\text{D}_2\text{O}$  plus the usual electrolyte but with no electrical current. The individual background runs were all featureless and closely followed the pattern of the integrated background shown in Fig. 2. Background rates in the neutron counter are  $\sim 10^{-3}\ \text{s}^{-1}$  in the energy region where 2.5-MeV neutrons are anticipated. By comparing energy spectra from  $\gamma$ -ray and neutron sources we have determined that approximately one-fourth of the observed background events arise from accidental coincidences of  $\gamma$ -rays and three-fourths from ambient neutrons. The  $\gamma$ -ray background comes mainly from radioactive radium and potassium in the surrounding materials.

We attribute the ambient neutrons to cosmic-ray sources. Although the typical neutron evaporation spectrum (at birth) has a broad maximum near 2.5 MeV (ref. 10), Monte Carlo calculations show that moderation in the source medium (predominantly the shielding surrounding the detector) will wash out this structure and produce a smoothly decreasing background spectrum above 0.5 MeV, as observed.

The predicted and measured absence of structure in the spectrum of cosmic-ray-produced neutrons will not be influenced by the relatively small temporal variations that may occur in the cosmic-ray flux, such as the observed decreases that may accompany solar flares. This means that the observed peak at 2.5 MeV cannot be accounted for by ambient-neutron background variations, because, as explained below, the analysis is based on the shape of the spectra and not simply on rates. Low-energy cosmic-ray muons would be rapidly scavenged by nuclei with high atomic number, so as to reduce muon-catalysed d-d fusion to a negligible level<sup>2,3</sup>. Considering volume and solid angle, the rate of production of neutrons by muons absorbed by carbon nuclei in the detector exceeds that from muons absorbed by oxygen nuclei in the electrolytic cells by a factor of  $\sim 60$ . Thus, the presence or absence of electrolytic cells is an unimportant perturbation in the background.

During the search for suitable catalytic materials, the following (unoptimized) prescription for the electrolytic cells evolved. It began with salts typical of volcanic hot springs and included electrode-metal ions. The electrolyte is typically a mixture of  $\sim 160\ \text{g}\ \text{D}_2\text{O}$  plus various metal salts in  $\sim 0.1\ \text{g}$  amounts each:  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{PdCl}_2$ ,  $\text{CaCO}_3$ ,  $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ ,  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ,  $\text{TiOSO}_4 \cdot \text{H}_2\text{SO}_4 \cdot 8\text{H}_2\text{O}$ , and a very small amount of  $\text{AuCN}$ . The pH is adjusted to  $\leq 3$  with  $\text{HNO}_3$ . All 14 runs reported here began with this basic electrolyte.

Titanium and palladium, initially selected because of their large capacities for holding hydrogen and forming hydrides, were found to be effective negative electrodes. Individual electrodes consisted of  $\sim 1\ \text{g}$  purified 'fused' titanium in pellet form, or  $0.05\ \text{g}$  of  $0.025\text{-mm}$ -thick palladium foils, or  $5\ \text{g}$  of mossy palladium. Typically 4–8 cells were used simultaneously. The palladium pieces were sometimes re-used after cleaning and roughening the surfaces with dilute acid or abrasives. Hydrogen bubbles were observed to form on the Pd foils only after several minutes of electrolysis, suggesting the rapid absorption of

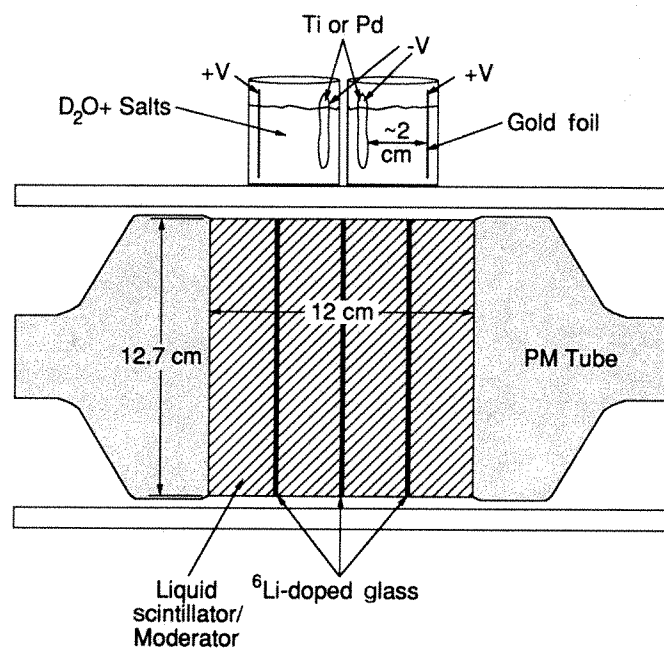


FIG. 1 Schematic diagram of the experiment. Electrolytic cells are shown on top of the neutron spectrometer.

deuterons into the foil; oxygen bubbles formed at the anode immediately. Gold foil was used for the positive electrodes. Direct-current power supplies provided 3–25 volts across each cell at currents of 10–500 mA. Correlations between fusion yield and voltage, current density, or surface characteristics of the metallic cathode have not yet been established.

Small jars, ~4 cm high and 4 cm in diameter, held ~20 ml of electrolyte solution each. The electrolytic cells were placed on or alongside the neutron counter, as shown in Fig. 1. The present cells are simple and undoubtedly far from optimum. Nevertheless, the present combination of our cells with the neutron spectrometer is sufficient to establish the phenomenon of cold nuclear fusion during electrolytic infusion of deuterium into metals.

Figure 2 shows the energy spectrum obtained under the conditions described above, juxtaposed with the (scaled) background spectrum. We acquired about twice as much background data as foreground data. Assuming conservatively that all deviations from background are statistical fluctuations, we scale the background counts by a factor of 0.46 to match the total number of foreground counts over the entire energy range shown in Fig.

A feature in channels 45–150 rises above background by nearly four standard deviations. This implies that our assumption is too conservative and that this structure represents a real physical effect. After re-scaling the background by a factor of 0.44 to match the foreground levels in regions just below and just above this feature, the difference plot (Fig. 3) is obtained. It shows a robust signal centred near channel 100, with a statistical significance of almost five standard deviations. A gaussian fit to this peak yields a centroid at channel 101 with a standard deviation of 28 channels, and an amplitude of  $23.2 \pm 4.5$  counts. Both the position and width of this feature correspond to those expected for 2.5-MeV neutrons, according to the spectrometer calibration. The fact that a significant signal appears above background with the correct energy for d–d fusion neutrons (~2.5 MeV) provides strong evidence that room-temperature nuclear fusion is occurring at a low rate in the electrolytic catalysis cells.

### Fusion rate determination

It is instructive to examine the fourteen individual runs which enter into the combined data discussed above. These runs were performed over the period 31 December 1988 to 6 March 1989. Figure 4 displays, for each run, the ratio of foreground count rate in the 2.5-MeV energy region to the background rate

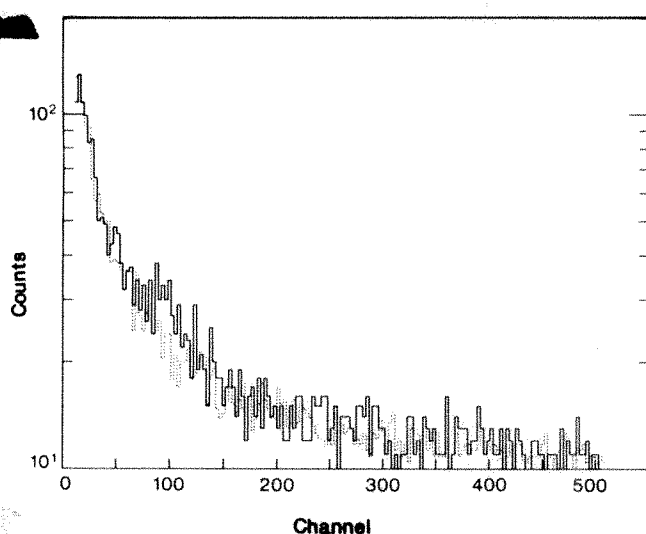


FIG. 2 Foreground (solid) and background (dashed) counts as a function of pulse height (corresponding to neutron energy) in the neutron spectrometer. Ten counts have been added to each three-channel bin for clarity of presentation.

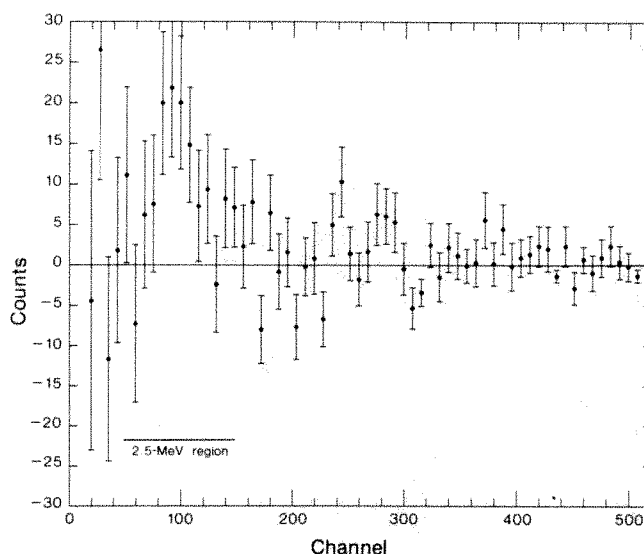


FIG. 3 Difference spectrum obtained by subtracting scaled background from the foreground. Statistical errors ( $\pm 1\sigma$ ) are shown for each eight-channel bin.

obtained for each run. Electronic changes were made in the apparatus during the course of the experiment which altered the observed background rates, so we plot the data in terms of foreground-to-background ratios rather than absolute rates. In one set of data (runs 1 to 8) for which the system was kept as untouched as possible to avoid changes in background rates, the measured rate of detection of 2.5-MeV neutrons was  $(6.2 \pm 1.3) \times 10^{-4} \text{ s}^{-1}$  above background. For this set of data, the background and foreground rates for all energies above ~3 MeV (that is, for all channels from 190 to 512) are equal, at  $(1.4 \pm 0.1) \times 10^{-3} \text{ s}^{-1}$ .

Run 6 is particularly noteworthy, with a statistical significance of approximately five standard deviations above background. Fused titanium pellets were used as the negative electrode, with a total mass of ~3 g. The neutron production rate increased after about one hour of electrolysis. After about eight hours, the rate dropped dramatically, as shown in the follow-on run 7. At this time, the surfaces of the titanium electrodes showed a dark grey coating. An analysis using electron microscopy with a microprobe showed that the surface coating was mostly iron, deposited with deuterons at the cathode. The same phenomenon of a decrease in the neutron signal after about eight hours of operation appears in run 13 followed by run 14. Runs 13 and 14 use the same eight electrochemical cells, and again the negative electrodes developed coatings after a few hours of electrolysis. These observations suggest the importance of surface conditions for the cold fusion process. Variations in surface conditions and electrolyte composition are anticipated during each test run because materials plate out of solution; the solution pH also changes significantly during a run. These 14 runs represent two choices of electrode material plus various operating currents. These variations may account for the fluctuations in the signal level that are evident in Fig. 4. As these runs represent a total of only ~200 signal neutrons at an average rate of ~2 per hour, it was difficult to optimize experimental conditions. This is a task for future research.

The observed 'turning off' of the signal after about eight hours may account for low signal-to-background ratios in runs 1 and 3, in that a signal that lasted for only a few hours may have been overwhelmed after a long (~20-hour) running time. When run 10 started with rates substantially above background, we stopped the run and removed half of the electrochemical cells as a test. The neutron production rate dropped off as expected (run 11). In determining the statistical significance of the data, we included runs 1, 3, 7, 11 and 14, even though we see a

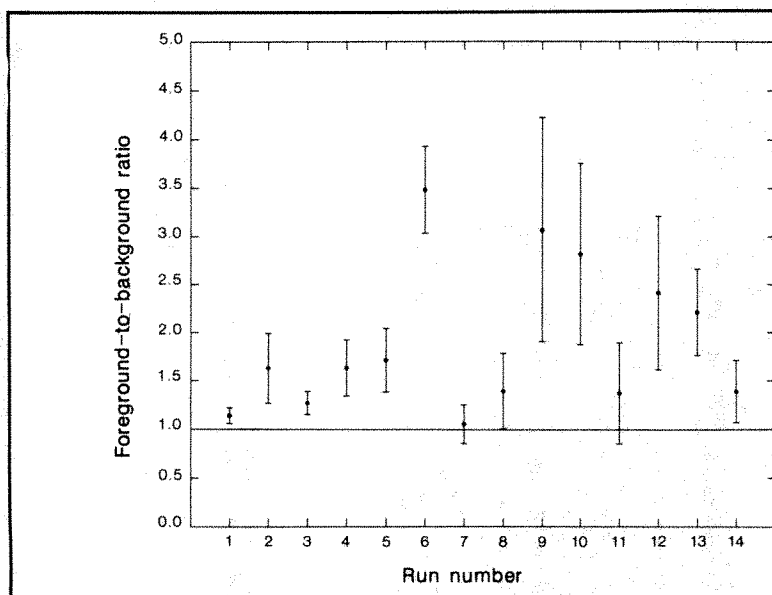


FIG. 4 Ratio of foreground rate to background rate for each run, in the 2.5-MeV energy region of the pulse-height spectrum. Statistical errors ( $\pm 1\sigma$ ) are shown.

systematic reason for their low foreground-to-background ratios as explained above. Run 8, shown in Fig. 4, was inadvertently lost from the magnetic storage device and could not be included in Figs 2 and 3. This does not change our conclusions.

We can estimate the rate for the neutron-production branch of d-d fusion during electrolysis, specifically for run 6, as follows:

$$\text{Fusions per deuteron pair per second} = \frac{R/\epsilon}{M \times \frac{d}{2M}} \quad (4)$$

where the observed rate of neutron detection,  $R = (4.1 \pm 0.8) \times 10^{-3} \text{ s}^{-1}$ , is based on foreground minus corresponding background counts in channels 45–150; the neutron detection efficiency, including geometrical acceptance, is calculated using a Monte Carlo neutron-photon transport code<sup>11</sup> to be  $\epsilon = (1.0 \pm 0.3)\%$ ;  $M \approx 4 \times 10^{22}$  titanium atoms for 3 g of titanium; and the ratio of deuteron pairs to metal ions,  $d/2M \approx 1$ , is based on the assumption that nearly all tetrahedral sites in the titanium lattice are occupied, forming the  $\gamma\text{-TiD}_2$  hydride. Then the estimated cold nuclear fusion rate for the neutron-production branch, by equation (4), is  $\lambda_f \approx 10^{-23}$  fusions per deuteron pair per second. If most fusions take place near the surface, or if the titanium lattice is far from saturated with deuterons, or if conditions favouring fusion occur intermittently, then the inferred fusion rate must be much larger, perhaps  $10^{-20}$  fusions per deuteron pair per second.

We note that such a fusion rate could be achieved by 'squeezing' the deuterons to about half their normal ( $0.74 \text{ \AA}$ ) separation in molecules. That such rates are now observed in condensed matter suggests catalysed 'piezonuclear' fusion as the explanation<sup>1</sup>. A possible cause is that quasi-electrons form in the deuterated metal lattice, with an effective mass a few times that of a free electron. Isotopes of hydrogen are known to accumulate at imperfections in metal lattices<sup>12</sup>, and a local high concentration

of hydrogen ions might be conducive to piezonuclear fusion. Because we have not seen any evidence for fusion in equilibrated, deuterated metals or compounds such as methylamine- $\text{d}_2$ , deuteriochloride or ammonium- $\text{d}_4$  chloride, we conclude that non-equilibrium conditions are essential. Electrolysis is one way to produce conditions that are far from equilibrium.

It may seem remarkable that one might influence the effective rate of fusion by varying external parameters such as pressure, temperature and electromagnetic fields, but just such effects are seen in another form of cold nuclear fusion, muon-catalysed fusion<sup>13</sup>.

## Conclusions

The correlation of ideas regarding cold piezonuclear fusion<sup>1</sup> with observations of excess  $^3\text{He}$  in metals and in geothermal areas of the Earth led to our experimental studies of fusion in electrochemical cells, which began in May 1986. Our electrolyte compositions evolved from geochemical considerations, and changed as results were observed. The presence of a fusion neutron signal was consistently reproduced, although the rate varied widely. Now that our exploratory searches have disclosed a small piezonuclear fusion effect, it remains to disentangle the factors that influence the fusion rate.

The need for off-equilibrium conditions is clearly implied by our data, and suggests that techniques other than electrochemistry may also be successful. We have begun to explore the use of ion implantation and of elevated pressures and temperatures, mimicking geological conditions. Cold nuclear fusion in condensed matter may be of interest as a novel probe of metal-hydrogen systems, including geological ones, and as a source of monoenergetic neutrons. If deuteron-deuteron fusion can be catalysed, then the d-t fusion reaction is possibly favoured because of its much larger nuclear cross-section. Although the fusion rates observed so far are small, the discovery of cold nuclear fusion in condensed matter opens the possibility, at least, of a new path to fusion energy. □

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■ For a comment from one of the referees of this paper, please see page 711.



# Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos

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Opposing anterior and posterior morphogen systems specify the segmented body pattern of *Drosophila*. The anterior morphogen, *bicoid*, exerts a direct, instructive influence on head and thoracic pattern by triggering different outcomes according to changes in its concentration along the body. In contrast, the posterior morphogen, *nanos*, simply defines where abdominal patterning can occur by eliminating an otherwise ubiquitous repressor, *hunchback* protein, from the posterior half of the embryo. Within this *hunchback*-free domain the pattern of abdominal segments must be specified by other morphogens, possibly by shorter range gradients of the products of zygotic gap genes *Kruppel*, *knirps* and *tailless*.

THE body plan of *Drosophila* is laid down in response to opposing morphogen gradients emanating from localized sources at each pole of the embryo<sup>1-3</sup>. The anterior morphogen, *bicoid* (*bcd*), appears to organize head and thoracic pattern by directly dictating the spatial expression of several subordinate signalling molecules<sup>4-7</sup>. In this regard, it behaves as a classical gradient morphogen, triggering a series of distinct results according to its changing concentration along the body<sup>8,9</sup>. Less is known about the posterior morphogen, *nanos* (*nos*)<sup>3,10,11</sup> although diffusion or transport of this molecule from the posterior pole towards the middle of the early embryo is normally essential for the development of abdominal pattern<sup>2</sup>.

The *bicoid* (*bcd*)<sup>1,3-7</sup> and *nanos* (*nos*)<sup>3,10,11</sup> morphogens profoundly influence the patterns of expression of many subordinate signalling molecules<sup>4-7,12-15</sup>. Although most of these effects are likely to be indirect, it is notable that both morphogens play early and major roles in generating restricted anterior expression of the protein product of the gap gene *hunchback* (*hb*; refs 12-14)<sup>5</sup>. The *hb* gene is first expressed during oogenesis and its transcripts are deposited throughout the egg<sup>17</sup>. Following fertilization, *nos* morphogen emanating from the posterior pole appears to destabilize these maternal transcripts, thereby preventing early expression of *hb* protein in the posterior half of the embryo<sup>5,17,18</sup>. Shortly thereafter, *bcd* morphogen spreading from the anterior pole triggers the transcriptional activation of the *hb* gene in the anterior half of the body<sup>5,9,18</sup>.

Zygotic activity of the *hb* gene under the control of the *bcd* gradient is critically required for the development of normal head and thoracic segments<sup>13,14</sup>; indeed it is sufficient for normal development to occur even in the absence of the maternal *hb* contribution<sup>13</sup>. In contrast, the role of the *nos* morphogen in repressing posterior *hb* expression is of unknown significance. The two experiments reported here test the significance of this interaction by determining what happens when the repression of *hb* is overridden or circumvented. In the first experiment, ectopic expression of the *hb* coding sequence in the posterior half of the body is shown to cause phenotypes which mimic those caused by absence of the posterior determinant system.

Conversely, the second experiment establishes that the requirement for the posterior morphogen can be obviated by inactivating maternally derived *hb* transcripts by other means. Taken together, these experiments establish that the *nos* morphogen influences the development of abdominal pattern primarily, if not exclusively, by precluding posterior *hb* expression. Hence, *nos* appears to have a permissive rather than an instructive role in generating the posterior pattern, providing a sharp contrast to the role of *bcd* in organizing anterior pattern.

## Results

**Ectopic *hb* expression causes *nos* mutant phenocopies.** Ectopic *hb* expression was generated by placing the coding sequence of the *hb* gene under the control of the *Drosophila hsp70* promoter<sup>19</sup>, integrating the hybrid *hsp70-hb* gene into the germ line via P-element mediated transformation<sup>20</sup>, and then activating transcription of the transformed gene by exposing embryos carrying it to heat shocks at various stages of their development (see legend to Fig. 1). As shown in Fig. 1*a-c*, heat shocking *hsp70-hb* embryos during the syncytial blastoderm stage generates a pattern of posterior *hb* expression similar to that of embryos lacking the posterior determinant system. When allowed to develop to hatching, such embryos give rise to larvae lacking or having severely reduced abdominal segmentation (Fig. 2*a-c*), a phenotype characteristic of larvae derived from embryos in which the *nos* morphogen is largely or completely inactive (for example, Fig. 2*d*)<sup>2,3,11</sup>. The loss of abdominal segmentation observed in both cases appears to arise in a similar fashion as indicated by the expression of the pair-rule gene *even-skipped* (*eve*)<sup>12,21,22</sup>. The periodic expression of this gene is one of the earliest manifestations of segmentation. As shown in Fig. 1*d-f*, heat-shocked *hsp70-hb* embryos show an altered pattern of *eve* expression which resembles that of embryos lacking the posterior determinant system.

Thus, driving inappropriate posterior expression of the *hb* protein in wild-type embryos is sufficient to cause phenotypes which mimic those associated with absence of the posterior determinant system. This result suggests that the *nos* morphogen may normally influence abdominal segmentation solely by destabilizing maternal transcripts of the *hb* gene, thereby precluding posterior expression of *hb* protein during early embryogenesis. If so, the posterior determinant system should only be required in embryos that receive functional maternal transcripts from the *hb* gene: embryos lacking such transcripts should develop normal abdominal patterns even if the *nos* morphogen is inactive. As described below, this prediction has been tested and confirmed by using X-ray-induced mitotic recombination to remove functional *hb* transcripts from single cells during the development of the female germ line<sup>23,24</sup>.

**The *nos* morphogen is superfluous in embryos lacking functional maternal *hb* transcripts.** Females homozygous for mutations in the *vasa* gene give rise to eggs in which the *nos* morphogen is inactive (refs 11, 25; Fig. 2*d*). These eggs also lack functional germ-cell determinants normally deposited at the posterior pole and hence give rise to agametic embryos. As shown in Fig. 3, it is possible to remove *hb* gene function from single germ cells in *vasa* females by irradiating larvae of the genotype *vas/vas*; *hb/+*. Of approximately 800 *vas/vas*; *hb/+* females irradiated



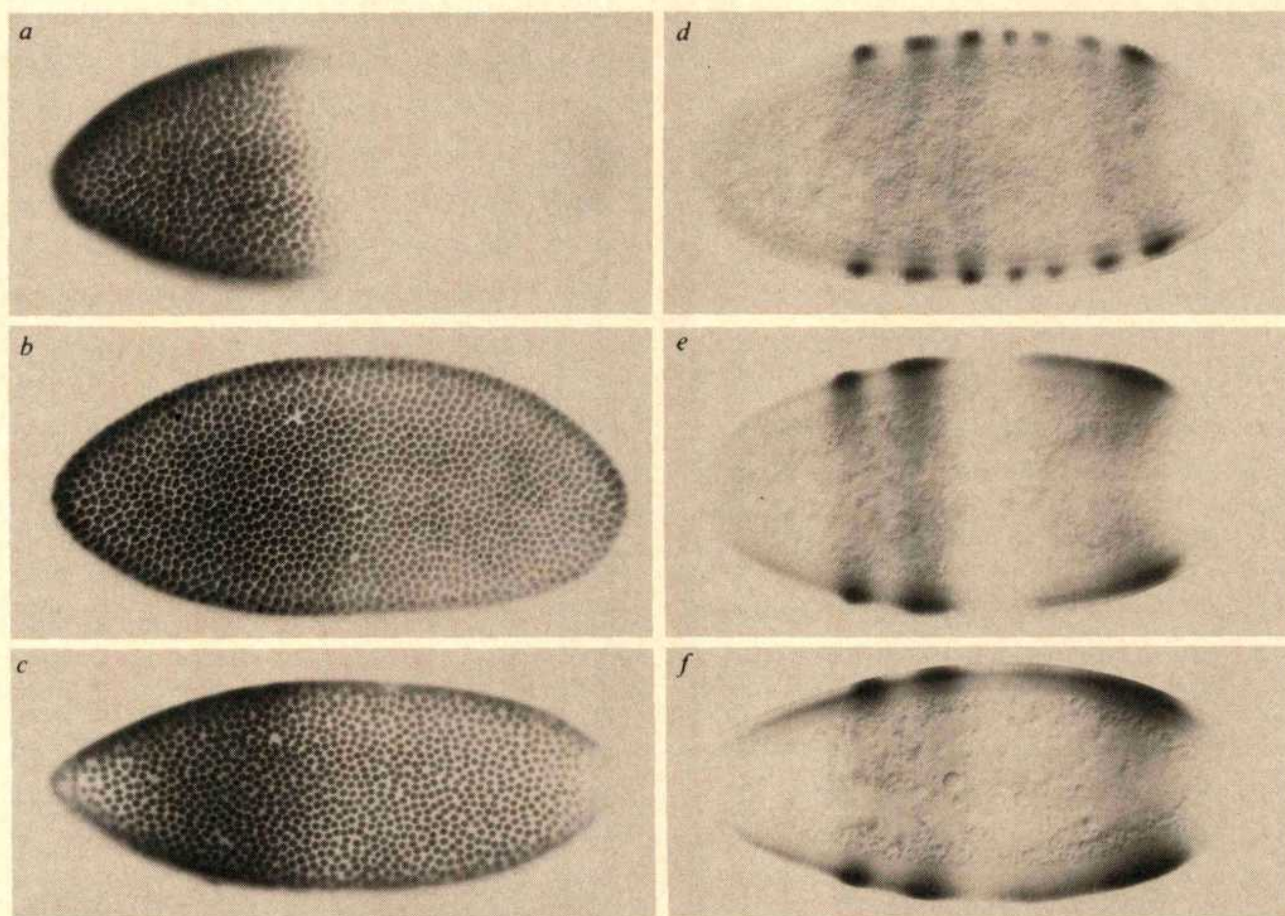


FIG. 1 Ectopic expression of the *hunchback* protein under *hsp70* control mimics the phenotype associated with absence of the posterior determinant *nanos*. *a* and *b*, Expression of *hb* protein in wild-type and *hsp70-hb* embryos 20 minutes after heat shock (15 min, 36 °C). *c*, Expression of *hb* protein in embryos derived from *vasa* females, which lack *nos* function (all embryos are in the twelfth nuclear division cycle following fertilization and are oriented with their anterior poles at the left; *vasa* mutant embryos were obtained from females homozygous for the *vasa*<sup>PD</sup> mutation). Note that wild-type embryos express *hb* protein exclusively in the anterior half (under the control of the anterior morphogen, *bcd*), whereas heat-shocked *hsp70-hb* and *vasa* embryos show ectopic expression of *hb* protein in the posterior half. *d*, *e* and *f*, Expression of *eve* protein in wild-type and *hsp70-hb* embryos 60 minutes after heat shock (10 min, 36 °C), and in *vasa* embryos, respectively (all embryos are in the later portion of the fourteenth nuclear division cycle, just before completing cellularization and beginning gastrulation, and are oriented as in *a*, *b* and *c*). The protein product of the *eve* gene is normally expressed in seven stripes at this stage (numbered 1–7 in the anterior to posterior direction). In both heat-shocked *hsp70-hb* and *vasa* mutant embryos, stripes 1 and 2, which are normally associated with the gnathal and anterior thoracic segments, are present, but broader than normal; however, stripes 3–6, which are normally associated with posterior thoracic segments and the first seven abdominal segments appear to be replaced by a domain of intermediate expression which seems to be fused to an enlarged stripe 7. The extent of this broad posterior domain is variable in both heat shocked *hsp70-hb* embryos as well as in *vasa* mutant embryos, although as shown here, the overall pattern is generally similar. Note that the extent of ectopic *hb* expression under *hsp70* control is variable: the embryo in *b* shows a typical example, although *hsp70-hb* expression is often so strong that the embryos appear to express *hb* protein uniformly. Note also that heat shocks administered any time after the blastoderm stage have no detectable effects on the pattern of body segments, even though high levels of ectopic *hb* expression are induced and persist for several hours.

**METHODS.** The *hsp70-hb* gene was constructed as follows: a 2.4-kb *Xba*I fragment from the *hb* gene containing the entire *hb* coding sequence

preceded by 15 nucleotides at the 5' end and followed by 152 nucleotides at the 3' end was excised from a genomic clone *pE8-B1000A* (kindly supplied by D. Tautz: ref. 17), and inserted into an *hsp70* expression plasmid such that it is positioned immediately downstream of the *hsp70* promoter<sup>19,29</sup> and upstream of the 3' untranslated portion of the  $\alpha$ 1 tubulin gene (including a putative transcriptional stop site (see ref. 30)). The resulting *hsp70-hb-tubulin* hybrid gene was then excised and inserted into the *ry*<sup>+</sup> P-element vector *Carnegie 20* (ref. 31) and integrated into the genome by P-element mediated transformation<sup>20</sup>. Two transformants were obtained, one of which, *HSHB-3*, contains an insert on the third chromosome. All of the experiments were performed using a stock which is homozygous for this insert. Embryos were heat-shocked as follows. Approximately staged embryos were obtained from timed egg collections, rinsed on a piece of nytex, and the nytex placed on the wet surface of an agar plate maintained at 36 °C. After 10 or 15 min (heat shocks of either duration gave indistinguishable results) the nytex was transferred to a fresh wet agar plate at 25 °C and incubated at that temperature until they were fixed for antibody staining or examined for their cuticular phenotypes (see Fig. 2). Embryos were stained for *hb* or *eve* protein as previously described<sup>32</sup> using rat anti-*hb* and rat anti-*eve* polyclonal antisera generously provided by P. Macdonald. Virtually all *hsp70-hb* embryos stained for *hb* antigen following heat shock show moderate to high levels of uniform *hb* expression superimposed on top of the normal pattern of *hb* expression (note however that embryos heat-shocked before the syncytial blastoderm stage do not show ectopic *hb* expression; also, ectopic *hb* expression was not observed in pole cells). The expression of *hb* protein induced by heat shocking *hsp70-hb* embryos increases progressively over a 1–2 hr interval following a 10 or 15-min heat shock, and slowly dissipates over a period of several hours thereafter. Most (> 90%) of the *hsp70-hb* embryos in the late phases of nuclear cycle 14 obtained following a 10-min heat shock 60 minutes earlier show abnormal patterns of *eve* expression similar to that shown in *e*, the principal variation being the extent of the posterior domain of expression (in a minority of cases, this broad domain appeared to partially resolve into two neighbouring domains). Older sibling embryos obtained in the same collections showed normal *eve* patterns of expression and segmental morphologies.



during the first or second larval instars, at least 24 gave rise to normal larval and adult progeny when outcrossed to wild-type males. As described in the legend to Fig. 3, all of the adult progeny from these females were shown to derive from *vas/vas*; *hb/hb* germ-line clones. Thus, *vasa* mutant embryos, which lack functional *nos* morphogen, can nevertheless develop into larvae and adults having normal abdominal patterns provided that they lack functional maternal transcripts of the *hb* gene.

## Discussion

The results presented here show that the primary, if not sole, role of the posterior determinant system is to prevent posterior expression of *hb* protein from maternal transcripts deposited throughout the egg. Similar conclusions have been reached independently by D. Tautz and colleagues (personal communication) on the basis of complementary experiments. These findings have several implications (see ref. 26 for a more extensive discussion of these points):

(1) Molecular role of the *nos* morphogen. The early and potentially exclusive role played by the *nos* morphogen in clearing maternal *hb* transcripts from the posterior half of the body suggests that it directly binds and destabilizes these transcripts. Both the *hsp70-hb* hybrid gene reported here, as well as an *hb-βgal* hybrid gene generated by Tautz *et al.* (personal communication) appear to be unaffected by the *nos* morphogen.

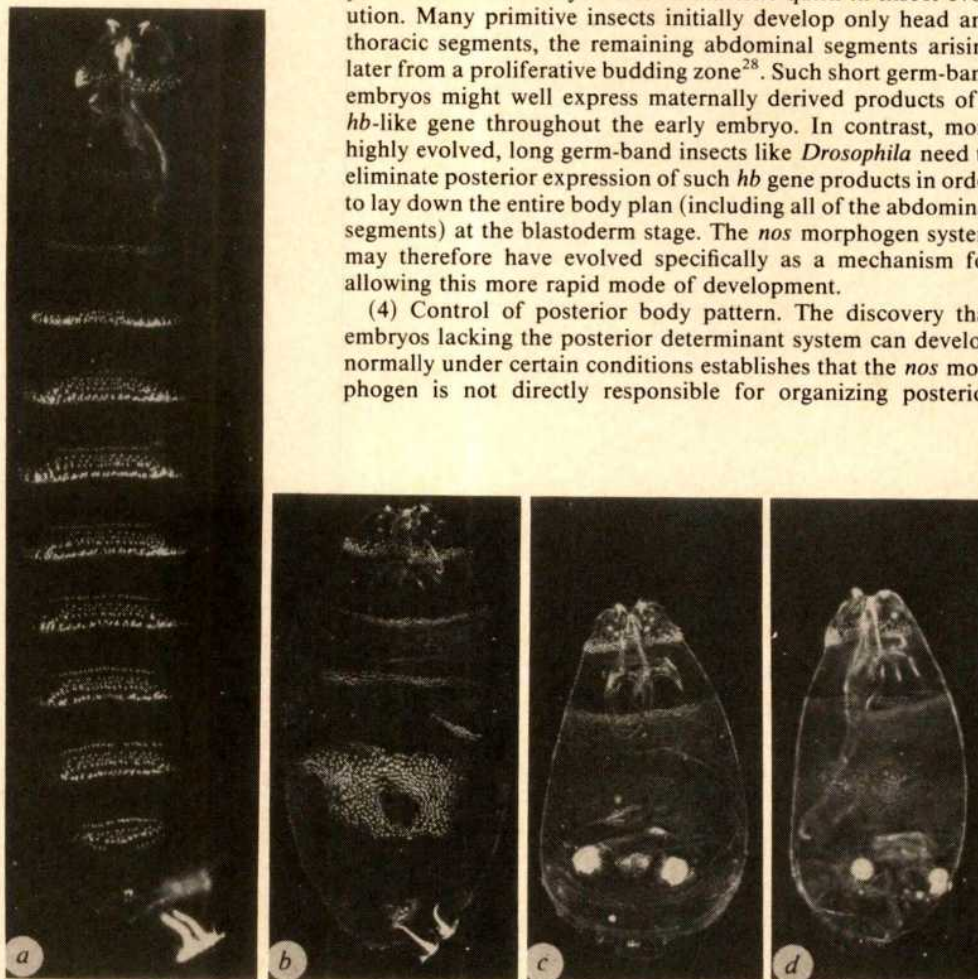
Transcripts from both of these hybrid genes lack most or all of the 3' untranslated portion of the endogenous *hb* transcript, suggesting that the target site for the *nos* morphogen may lie in this portion of the molecule.

(2) Molecular role of the *hb* protein. The posterior determinant is thought to control abdominal segmentation by controlling the regional activation of the gap gene *knirps* (*kni*)<sup>3,11</sup>. This supposition was initially based on the phenotype of *kni* mutant embryos that resembles that associated with mutations which partially inactivate the *nos* morphogen, and is supported by preliminary molecular studies of U. Nauber and colleagues (unpublished data, cited in refs 15, 27). The results presented here suggest that the *nos* morphogen activates *kni* gene expression indirectly by repressing inappropriate *hb* expression in the posterior half of the body. The *hb* protein may therefore directly repress transcription of the *kni* gene. Thus, the *bcd* and *nos* morphogens may specify the subdivision of the body into distinct anterior (*hb-on*; *kni-off*) and posterior (*hb-off*; *kni-on*) patterning domains by their immediate effects on the pattern of *hb* expression, the activation of *kni* resulting by default from the absence of *hb* protein.

(3) Role of maternal *hb* expression. The present results show that maternal transcripts of the *hb* gene are not simply dispensable in normal development<sup>13</sup>—they are potentially deleterious. Indeed, failure to remove these unwanted transcripts from the back half of the embryo prevents abdominal differentiation. This peculiar situation may reflect an atavistic quirk in insect evolution. Many primitive insects initially develop only head and thoracic segments, the remaining abdominal segments arising later from a proliferative budding zone<sup>28</sup>. Such short germ-band embryos might well express maternally derived products of a *hb*-like gene throughout the early embryo. In contrast, more highly evolved, long germ-band insects like *Drosophila* need to eliminate posterior expression of such *hb* gene products in order to lay down the entire body plan (including all of the abdominal segments) at the blastoderm stage. The *nos* morphogen system may therefore have evolved specifically as a mechanism for allowing this more rapid mode of development.

(4) Control of posterior body pattern. The discovery that embryos lacking the posterior determinant system can develop normally under certain conditions establishes that the *nos* morphogen is not directly responsible for organizing posterior

FIG. 2 Cuticular phenotypes of larvae derived from wild-type heat shocked *hsp70-hb* and *vasa* embryos. Dark-field micrographs showing the ventral aspects of larvae derived from wild-type embryos (a), heat shocked *hsp70-hb* embryos (b and c) and *vasa*<sup>PD</sup> mutant embryos (d) (larvae in a and b show only the ventral aspect; larvae in c and d show superimposed ventral and dorsal aspects; all embryos are oriented with their heads at the top). Wild-type larvae differentiate eight abdominal segments which are associated with distinctive patterns of thick ventral hairs (dentical bands). These are preceded anteriorly by three thoracic segments associated with bands of fine ventral hairs and the head which includes prominent derivatives of the mouthpart segments, and followed posteriorly by terminal structures including the paired spiracles. *hsp70-hb* embryos heat-shocked approximately 105–165 min after fertilization give rise to larvae which have normal heads and anterior thoracic segments but lack most or all of the abdominal segments as shown in b and c. (Single collections of heat shocked embryos were split into two populations, one assayed for eve protein expression 60 minutes after heat shock, the other analysed for cuticular pattern following differentiation. Of the embryos which differentiate larval cuticle, more than 50 per cent show segmental patterns which range between the embryos shown in b and c; larvae showing wild-type cuticular patterns almost certainly developed



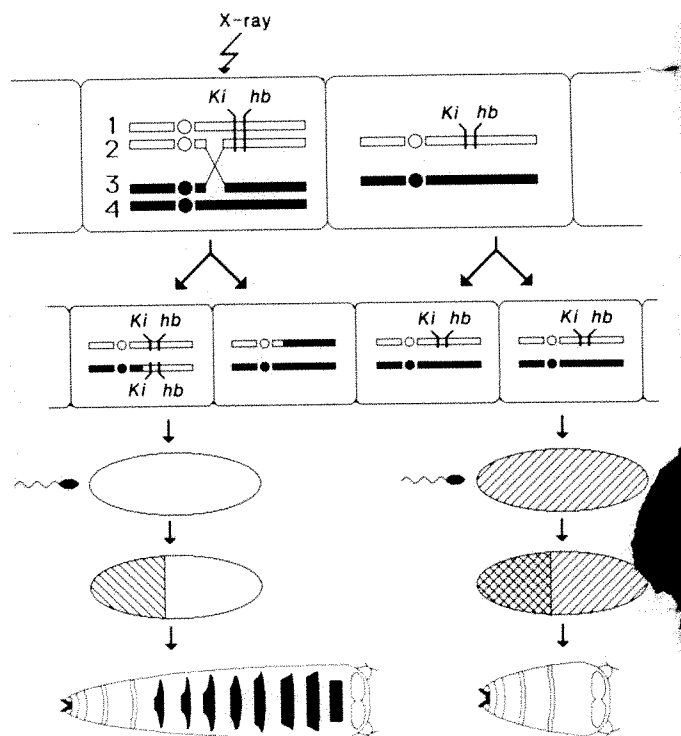
from embryos heat shocked towards the end of the blastoderm stage or later (which show normal patterns of eve expression.) In larvae lacking all abdominal segments, the third thoracic segment is often partially deleted and the posterior spiracles are rudimentary and fail to pair, as shown in c. These larvae closely resemble larvae derived from *vasa* mutant embryos (d) which generally lack all of the abdominal segments, show partial deletions

of the third thoracic segment and have unpaired, rudimentary spiracles.

**METHODS.** Unhatched larvae were dechorionated with commercial bleach, transferred to a mixture of 1:1 heptane:methanol and vortexed until most were separated from the vitelline membrane. They were then rinsed in 0.1% Triton-X and mounted in a mixture of 1:1 Hoyer's mountant:lactic acid<sup>33</sup>.



**FIG. 3** Induction of homozygous *hb* clones in the germ lines of females lacking the posterior determinant system. Female first and second instar larvae of the genotype *vas<sup>PD</sup>/vas<sup>PD</sup>; Ki hb<sup>14F</sup>/+* were irradiated with 1,000 rad to induce mitotic recombination in single germ cells as shown at the top. The *Kinked* (*Ki*) mutation is a dominant bristle marker which is positioned just proximal to the *hb* mutation on the right arm of chromosome 3 (ref. 34). Recombination between the *Ki* locus and the centromere will result in a homozygous *vas<sup>PD</sup>/vas<sup>PD</sup>; Ki hb<sup>14F</sup>* daughter cell, provided that chromatids 1 and 3 segregate together (the *Ki* and *hb* loci are tightly linked; hence, few if any recombination events are expected to occur between them). The descendants of this cell will form a germ-line clone giving rise to eggs lacking functional maternal transcripts of the *hb* gene. The other daughter cell will be homozygous for the wild-type *Ki* and *hb* alleles and, like the surrounding *vas<sup>PD</sup>/vas<sup>PD</sup>; Ki hb<sup>14F</sup>/+* cells, will give rise to eggs with ubiquitous, functional *hb* transcripts. Fertilization of *vas* eggs containing functional *hb* transcripts should generate phenotypically mutant embryos because the *vas* mutation prevents activity of the posterior determinant *nos*, thereby allowing the maternal *hb* transcripts (hatching) to persist and give rise to inappropriate posterior expression of the *hb* protein, as shown. Conversely, removing the wild-type allele of *hb* from the germ line via mitotic recombination should abolish this inappropriate posterior expression, whereas zygotic activation of the paternal *hb* gene (opposite hatching) under the control of the *bcd* gradient should suffice to generate the necessary anterior domain of *hb* expression. Note that the *vas<sup>PD</sup>* mutation also prevents the formation of posterior polar granules associated with localized germ-cell determinants; consequently all of the progeny from *vas<sup>PD</sup>/vas<sup>PD</sup>; Ki hb<sup>14F</sup>/+* females are agametic, irrespective of the presence or absence of functional maternal *hb* transcripts. Note also that virtually all larvae derived from these females show a less extreme mutant phenotype than that observed in larvae derived from *vas<sup>PD</sup>/vas<sup>PD</sup>* mothers which carry two functional copies of the *hb* gene. In particular, they generally form a single band of abdominal denticles between the thorax and posterior terminalia in contrast to standard *vas<sup>PD</sup>* mutant embryos (for example, Fig. 2d) which do so only occasionally. This slight degree of phenotypic rescue probably results directly from the lower maternal *hb* gene dosage in *vas<sup>PD</sup>/vas<sup>PD</sup>; Ki hb<sup>14F</sup>/+* females, as completely eliminating the maternal *hb* contribution, as described below, fully rescues abdominal segmentation, as shown. Approximately 800 female flies obtained from irradiated *vas<sup>PD</sup>/vas<sup>PD</sup>; Ki hb<sup>14F</sup>/+* larvae were outcrossed in small groups of 10–20 flies to wild-type males. Of the 70 such group matings which were performed, 24 gave rise to small numbers of wild-type larvae which developed in adult flies with normal abdominal patterns; all the rest produced only *vas* mutant larvae (nine of these 24 matings gave rise to single surviving flies, six gave rise to 2–3 survivors, and eight gave rise to four or more survivors). A total of 71 such adult flies were obtained: all were phenotypically *Ki* as was to be expected if they arose from mitotic recombination. In addition, all of the flies which survived



long enough to be tested (55/71) were sterile, and in fact agametic, as was to be expected if they arose from *vas* mutant eggs. Finally, in two cases it was possible to identify the single female fly generating phenotypically wild-type larvae; in both cases, all but the few surviving progeny developed as *vas* mutant larvae. The phenotypic properties of the surviving flies therefore establish that these progeny arose from homozygous *Ki hb<sup>14F</sup>/Ki hb<sup>14F</sup>* clones in the germ lines of their homozygous *vas<sup>PD</sup>/vas<sup>PD</sup>* mothers. **METHODS.** Surviving flies were tested for fertility by outcrossing them singly to flies of the opposite sex for 5–7 days. Their ovaries or testes were then removed and examined under phase and Nomarski optics for the presence of germ cells. The frequency of clones obtained (~1 clone per 25 flies) is similar to that obtained in other studies (see for example, ref. 24); given that 24/70 group matings produced surviving adults, it is likely that more than one female contained a clone in some of these matings.

pattern. Instead, activity of the *nos* morphogen appears only to define the region in which abdominal segmentation will occur. Other spatial determinants must therefore be responsible for organizing pattern within this domain. One possibility is that the overlapping distributions of the products of the gap genes *kruppel*, *kni* and *tailless*<sup>6,12,16,27</sup> provide a series of short-range

gradients which govern the subdivision of the posterior half of the embryo into particular abdominal segments. This strategy of controlling body pattern would differ significantly from the strategy operating in the anterior half of the embryo where the graded distribution of a single molecular species, *bcd*, provides a coherent system of cues organizing the global pattern. □

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# Detectability of gravitational microlensing in the quasar QSO2237+0305

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THE quasar QSO2237+0305 is unique in being located almost exactly behind the centre of a bright nearby galaxy, of magnitude 14.5 and redshift 0.039 (ref. 1). Recent observations<sup>2-4</sup> show that the quasar is split into four images with very similar spectra, strongly suggesting gravitational lensing by the foreground galaxy. We show here that compact objects within the galaxy, such as stars, lying near the line of sight, can cause transient microlensing effects. Rapid changes owing to transverse motions of quasar, galaxy and observer turn out to be more likely in QSO2237+0305 than in any other system. The time delays between the four macro-images are about a day or less<sup>3</sup>, so intrinsic variations should show up 'simultaneously' in all images, and a change in observed luminosity ratios would be direct proof of microlensing. QSO2237+0305 is the only known system where such unequivocal proof is possible, making it a key object in assessing the general importance of microlensing.

Theoretical models<sup>3,5</sup> that assume a smooth distribution of matter in the foreground galaxy fit the observations fairly well, and we have developed a method whereby microlensing effects from compact objects near the line of sight can be estimated from the macrolensing model. For reasonable choices of system parameters and a quasar radius  $R_q < 0.05 (M/M_\odot)^{1/2}$  pc, where  $M$  is the mass of the object causing microlensing, we expect changes larger than  $0.05 (M/M_\odot)^{1/2}$  mag per year in at least one of the images. For  $R_q < 0.005 (M/M_\odot)^{1/2}$  pc, high-amplification events should occur about once every three years in one of the images, and would lead to a microlensing amplification of more than a factor of two on a timescale of one year or less.

Microlensing by stars close to the light path has been discussed intensively over the past decade<sup>6-13</sup>. It is possible that gravitational lensing, and particularly microlensing, is affecting a large fraction of the distant quasars. If so, our concepts of quasar structure, quasar evolution, quasar statistics, and mass distribution in the Universe (abundance of compact masses between  $10^{-3} M_\odot$  and  $10^3 M_\odot$ ) must clearly be revised. From the observational point of view there are some indications of variability arising from microlensing (ref. 14, C. Vanderriest *et al.*, preprint, and R. E. Schild, personal communication), but there is no firm evidence at present. To assess the general importance of microlensing, the most promising systems for such effects should be investigated first. These systems are the gravitationally lensed quasars with several macro-images, for which enough mass is 'between' the images<sup>15</sup> that a large optical depth for microlensing ( $\tau$ ) usually results. Typically one finds a  $\tau$  larger than  $0.5 \epsilon$  in some of the images, where  $\epsilon$  is the fraction of the deflector mass that is in stars. Furthermore, the discrimination between intrinsic and microlensing variability is much simpler in such systems.

Among the lensed quasars with several macro-images the quadruple QSO2237+0305 (refs 2, 3) is the most promising system, because the deflector is about ten times closer than in other known systems. Therefore the Einstein radius  $\zeta_0$  of a single star with mass  $M$  in the deflecting galaxy, projected into the source plane, is (with redshift of the deflector  $z_d = 0.039$ , redshift of the quasar  $z_q = 1.7$ , Hubble constant  $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$  and cosmological deceleration parameter  $q_0 = 0$ )

$$\zeta_0 \approx 0.05 \sqrt{M/M_\odot} \text{ pc} \quad (1)$$

which is about three times larger than for other known lens systems. As smearing effects strongly reduce the effect of microlensing when the quasar radius  $R_q$  is larger than  $\zeta_0$ , such effects are much less probable for QSO2237+0305. Also favourable is the fact that we expect a much larger transverse velocity  $V$  between the (anti-)caustics and the source because of the large distance ratio ( $D_q/D_d \approx 11$ ). We find that

$$V = \frac{v_q}{(1+z_q)} - \frac{D_q}{D_d} \frac{v_d}{(1+z_d)} + \frac{D_{dq}}{D_d} \frac{v_{\text{obs}}}{(1+z_d)} \quad (2)$$

where  $D_d$ ,  $D_q$  and  $D_{dq}$  are apparent size distances and  $v_d$ ,  $v_q$  and  $v_{\text{obs}}$  are the transverse velocities involved. With  $v_d$  and  $v_{\text{obs}} \approx 400 \text{ km s}^{-1}$  we expect  $V \approx 6,000 \text{ km s}^{-1}$ , which is about a factor of 10 larger than for other known systems. This results in a reduced characteristic timescale for microlensing of

$$\Delta t_0 = \zeta_0/V \approx 8 \sqrt{M/M_\odot} \frac{6,000 \text{ km s}^{-1}}{V} \text{ yr} \quad (3)$$

and more rapid changes in the observed luminosity of individual images. For  $R_q \leq 0.1 \zeta_0$ , that is,  $R_q < 0.005 \sqrt{M/M_\odot} \text{ pc}$ , a pronounced asymmetric peak in the light curve occurs when the source crosses a (micro-) caustic. For such high-amplification events (HAEs) we expect a maximum amplification owing to microlensing of

$$A_{\text{max}} \approx \sqrt{\zeta_0/R_q} \approx 2.5 (M/M_\odot)^{1/4} (0.005 \text{ pc}/R_q)^{1/2} \quad (4)$$

on a timescale of

$$\Delta t_h = R_q/V \approx (R_q/0.005 \text{ pc})(6,000 \text{ km s}^{-1}/V) \text{ yr} \quad (5)$$

With macro-lens modelling (homogeneous deflector) it is possible to determine the projected surface mass density  $\sigma_i$  and the shear parameter  $\gamma'$  at all image positions. To estimate microlensing effects, however, we need to know the normalized surface density  $\sigma$  in stars<sup>10,11</sup> or equivalently the optical depth  $\tau$  for microlensing<sup>16</sup>,

$$\sigma = \frac{\sigma_s}{1 - \sigma_c} = \tau \quad (6)$$

and the normalized shear parameter<sup>11</sup>

$$\gamma = \frac{\gamma'}{1 - \sigma_c} \quad (7)$$

Here  $\sigma_s$  is the projected smoothed-out surface mass density in stars, and  $\sigma_c$  is the projected continuous surface mass density, both in units of the critical surface mass density  $\sigma_0$ :

$$\sigma_0 = \frac{c^2}{4\pi G} \frac{D_q}{D_d D_{dq}} \quad (8)$$

With these parameters we can write the lens equation, which defines the mapping from the deflector plane onto the observer (or source) plane, in the normalized form (see ref. 11 for a more detailed discussion)

$$\zeta = \text{sign}(\sigma) S(z) + \begin{pmatrix} 1+\gamma & 0 \\ 0 & 1-\gamma \end{pmatrix} z \quad (9)$$

where the first term represents the effect of stars close to the light path and the second term represents the large-scale field of the deflector as a whole. Note that the length unit in the source plane is now the projected Einstein radius (compare equations (1)). If the stars had been smeared out, the amplification of one macro-image would have been

$$A_G = |(1 - \sigma_c)^2 (1 - \sigma + \gamma)(1 - \sigma - \gamma)|^{-1} \quad (10)$$

Assuming a fraction  $\epsilon$  of the mass to be contained in stars ( $\sigma_s = \epsilon \sigma_i = \epsilon (\sigma_s + \sigma_c)$ ) we can calculate  $\sigma$  and  $\gamma$  from equations (6) and (7) if  $\sigma_i$  and  $\gamma'$  are known. By eliminating  $\epsilon$ , we find a simple relation between  $\sigma$  and  $\gamma$ :

TABLE 1 Assumed values for  $\sigma_i$  and  $\gamma'$  for the four images

	A	B	C	D
$\sigma_i$	0.36	0.45	0.88	0.61
$\gamma'$	0.44	0.28	0.55	0.66

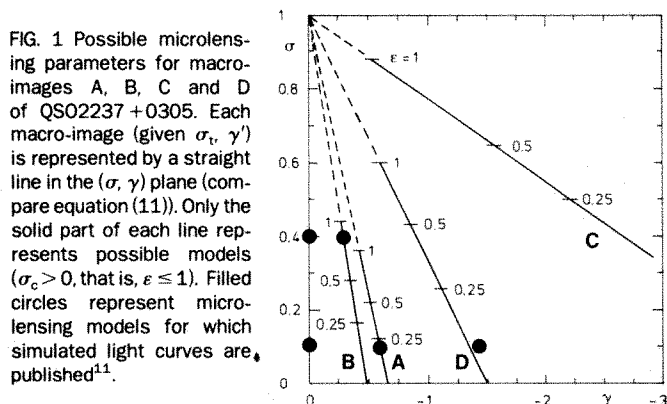
$$\sigma = 1 + \frac{\sigma_i - 1}{\gamma'} \gamma \quad (11)$$

For a given macro-image (given  $\sigma_i$ ,  $\gamma'$ ), this represents a straight line in the  $(\sigma, \gamma)$  diagram.

Theoretical macrolensing models of QSO2237+0305 which agree well with the observations have been presented recently by several groups<sup>3,5</sup>. The quantities  $Amp_{cal}$  and  $\tau_{lens}$  used in ref. 3 correspond to  $A_G$  and  $\sigma$  used in this paper (compare equation (10) and ref. 11). It is now a simple task to determine  $\gamma'$  (and  $\sigma_i$ ) for all four images, and the values are given in Table 1. We have here made use of the fact that images C and D have negative parities ( $\sigma_i + |\gamma'| > 1$ ) and that images A and B therefore must have positive parities. Note that the above discussion uses differently orientated coordinate systems for all four images.

The determination of  $\sigma_i$  and  $\gamma'$  is not possible from the data given in ref. 5 because  $\tau_{lens}$  is not given; because of the similarity of the solutions, however, we expect similar parameter values. Our test calculations also indicate that the parameter values based on ref. 3 are reasonably accurate, and we therefore adopt the values given in Table 1.

In Fig. 1 the corresponding  $\sigma(\gamma)$  relations (compare equation (11)) are shown for all images, with  $\epsilon$  values indicated for some



selected points. All the straight lines go through the point  $\sigma = 1$ ,  $\gamma = 0$ ; note, however, that only the solid parts of the lines correspond to possible models ( $\sigma_c > 0$ , that is,  $\epsilon \leq 1$ ). Also shown in Fig. 1 are models, with equal masses in all stars, for which light curves have been presented in ref. 11. Three of these models ( $\sigma, \gamma$ ) = (0.1, -0.6), (0.4, -0.4) and (0.1, -1.4), which were denoted A3, A4 and B1 respectively in ref. 11—are of particular interest here as they are very close to possible microlensing models of one of the images. The two models with  $\sigma = 0.1$  should yield a reliable lower limit to microlensing variability of images A, B and D, because their  $\epsilon$  values (0.17 and 0.08) are smaller than expected in reality, and the variability increases with increasing  $\epsilon$  for a given  $\sigma_i$  and  $\gamma'$ . This lower limit should also apply for image C, as it has a much larger  $\sigma$  than the other images for the same  $\epsilon$ .

From the 'normalized' light curve of the two  $\sigma = 0.1$  models presented in ref. 11, we find, for about 50% of the time, a rate of change larger than 0.2 mag per normalized unit  $\zeta_0$  for all source sizes calculated, that is, for  $R_q$  equal to 0.011  $\zeta_0$ , 0.1  $\zeta_0$  and 0.9  $\zeta_0$  respectively. For about 30% of the time the rate of

change is even larger than 0.4 mag per normalized unit. For  $R_q > \zeta_0$  it is clear, however, that such rapid variations occur much less frequently with increasing  $R_q$ .

To obtain time-dependent light curves we need to know the time  $\Delta t_0$  taken to move transversely by one normalized length unit. We use the estimate given in equation (3) (note that a relatively small  $\sigma_c$  ( $\sim 0.3$ ) has been assumed; compare Table 3 in ref. 11), and we find that two macro-images are expected to vary at a rate greater than  $0.025 \sqrt{M_\odot/M}$  ( $V/6,000 \text{ km s}^{-1}$ ) magnitudes per year, and one of them at a rate greater than  $0.05 \sqrt{M_\odot/M}$  ( $V/6,000 \text{ km s}^{-1}$ ) magnitudes per year if  $R_q$  is smaller than  $0.05 \sqrt{M/M_\odot}$  pc. Such independent variations in the four images should not be difficult to demonstrate, because they would lead to variations of the observed luminosity ratios. This would be a proof of microlensing because intrinsic variations would show up 'simultaneously' in the four images as a result of the small time delay (about one day or even less<sup>3</sup>), and could not lead to variations in the observed luminosity ratios. Note that QSO2237+0305 is the only known system that offers the possibility of such an unequivocal proof.

From the simulated light curves in ref. 11 we estimate the frequency of high-amplification events to be  $N_{HAE} \approx 0.3 \sqrt{M_\odot/M}$  ( $V/6,000 \text{ km s}^{-1}$ ) per year in one of the images. Accurate observations of the luminosity during an HAE can give valuable information on the structure and size of the source<sup>13</sup>. QSO2237+0305 is the most promising object for such an investigation, because 1) smearing effects are less probable than in other systems, 2)  $N_{HAE}$  is larger and 3) intrinsic variability and microlensing can easily be separated in this system. Regular monitoring of QSO2237+0305 (on the order of once a week) should therefore be given high priority.  $\square$

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## Stratospheric nitric acid vapour measurements in the cold Arctic vortex: implications for nitric acid condensation

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STRATOSPHERIC nitric acid condensation has recently been proposed<sup>1,2</sup> to take place in the cold winter polar vortex, priming it for chlorine-catalysed ozone destruction. This idea has been developed subsequently<sup>3–8</sup>. Condensation, thought to proceed via a heterogeneous heteromolecular mechanism involving stratospheric water vapour and pre-existing  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$  aerosols, which serve as condensation nuclei, leads to solid nitric acid trihydrate.



(NAT;  $\text{HNO}_3 \cdot 3\text{H}_2\text{O}$ ) aerosols. It is expected<sup>1,2,8</sup> that the condensation temperature,  $T_c(\text{NAT})$ , is significantly greater than that of water-ice,  $T_c(\text{H}_2\text{O-ice})$ , given the stratospheric abundances of gaseous nitric acid and water and the thermodynamic properties of stratospheric aerosols<sup>8</sup>. Knowledge of  $T_c(\text{NAT})$  is important because it determines the spatial and temporal extent of NAT aerosols and hence possibly of polar ozone destruction. Here we report *in situ* measurements of the detailed height distribution of gaseous nitric acid in the cold arctic vortex, using a balloon-borne technique<sup>9-16</sup>, which offers a much better altitude resolution (30 m) than previous satellite measurements<sup>17,18</sup>. Our data set an upper limit to  $T_c(\text{NAT})$  of 195 K at 23 km, and are consistent with model predictions based on the present gaseous nitric acid data, typical water vapour abundances and thermodynamic data<sup>8</sup> of macroscopic NAT mixtures. However, our upper limit to  $T_c(\text{NAT})$  is markedly lower than some of the early model estimates<sup>1,2</sup>.

The ACIMS instrument used for these measurements has been described previously<sup>9-16</sup>. The present limit for gaseous nitric acid detection is about  $1 \times 10^7$   $\text{HNO}_3$  molecules per  $\text{cm}^3$ , far lower than typical stratospheric nitric acid concentrations at heights below 30 km ( $>1 \times 10^9 \text{ cm}^{-3}$ ). The accuracy of the gaseous nitric acid concentrations measured here is  $\pm 50\%$  and the precision is  $\pm 20\%$ . An important aspect of the experiment is the possibility of perturbation of the nitric acid measurements by NAT aerosols, which may evaporate inside the flow tube of our instrument. Careful simulation and atmospheric test measurements<sup>16</sup>, including also aircraft flights in the lower stratosphere, have shown that significant evaporation of stratospheric  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$  aerosols can be induced only by massive additional heating of the gas stream. In our present measurements, however, the gas temperature inside the flow tube exceeds the ambient gas temperature by not more than a few degrees, therefore ruling out extensive NAT aerosol evaporation. The measurements reported here were part of the CHEOPS 2 expedition, which took place in January-February 1988 in the Arctic, mainly at Esrange, about 50 km east of Kiruna in Northern Sweden. During this expedition we carried out three balloon flights. One of these (B-88-2; 1 February 1988) was performed under conditions of particularly low stratospheric temperature and the results of just this flight are reported here. The balloon reached a maximum altitude of 31 km and ACIMS measurements were made on ascent between 13 and 31 km and on descent between 31 km and 5 km.

Figure 1 shows the time evolution of stratospheric temperatures in the core of the polar vortex and over Esrange at the 30 hPa pressure level. The height distribution of the temperature, measured by a sensor mounted 5 m above the balloon gondola, is depicted in Fig. 2. The accuracy of the temperature measurement is about  $\pm 0.3$  K. The coldest layers (193 K) were found around 23 km and 26 km. The measured height distribution of nitric acid vapour is given in Fig. 3a along with previous satellite data. Our data, with a resolution of 30 m, are generally consistent with satellite infrared measurements, which offer an altitude resolution of only 3,000–5,000 m. Our data are somewhat higher than previous model predictions<sup>19,20</sup> based only on gas-phase chemistry (also shown in Fig. 3a). Our total vertical column nitric acid density of  $1.5 \times 10^{16} \text{ cm}^{-2}$  is consistent with previous ground-based column measurements performed with infrared techniques<sup>21-23</sup> in the winter polar vortex. Figure 3b shows a portion of the nitric acid profile (around 23 km) with the maximum possible altitude resolution. Interestingly, no pronounced local minimum is seen at the heights where the lowest temperatures occurred. Some fine structure is observed, which may also be due to transport effects. We conclude from our data that  $T_c(\text{NAT})$  must have been equal to or lower than 195 K. If the temperature falls below  $T_c(\text{NAT})$  by only 2 K one may expect<sup>8</sup> gaseous nitric acid to become depleted by about a factor of four. We cannot exclude the possibility that some nitric acid condensation had already taken place, but we can be sure that  $T_c(\text{NAT})$  is no more than 2 K greater than the measured  $T$ .

Three days before our balloon flight, a search for stratospheric layers with enhanced aerosol backscattering was carried out by Poole *et al.*<sup>24</sup> using an aircraft-borne lidar (laser radar) (Fig. 1). On this flight, enhanced lidar-backscattering was observed only close to the centre of the vortex between Spitsbergen and Greenland, where the lowest temperature around 23 km was  $\sim 190$  K. The observed excess aerosol layer was located around 23 km and was very thin (300 m).

Using the nitric acid measurements, we may estimate the height variation of  $T_c(\text{NAT})$ , on the basis of laboratory measurements<sup>8</sup> of macroscopic NAT mixtures and taking for the stratospheric abundance of water vapour a typical value of 5 p.p.m.v. (as measured by satellite-borne remote-sensing techniques<sup>25</sup> in the winter Arctic vortex around 20 km altitude). The resulting

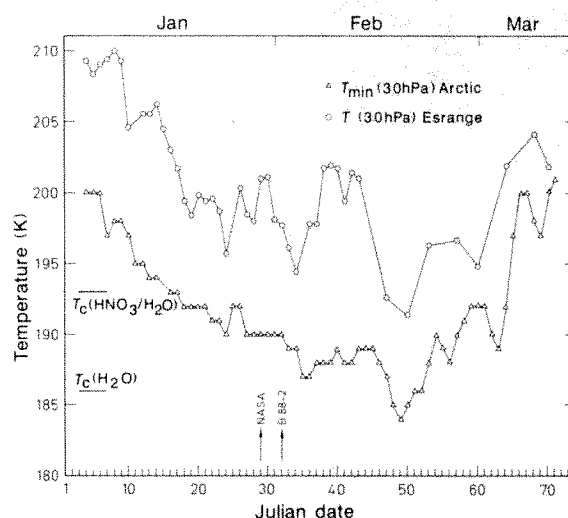


FIG. 1 Time evolution of stratospheric temperatures in the Arctic during winter and early spring of 1988. Condensation temperatures at 30 hPa for pure  $\text{H}_2\text{O-ice}$  and NAT are indicated. Also marked are dates of our balloon flight B88-2 and the NASA aircraft flight. See text for details. The Arctic temperatures were provided by the Meteorological Institute of the Free University of Berlin (K. Labitzke *et al.*, personal communication).

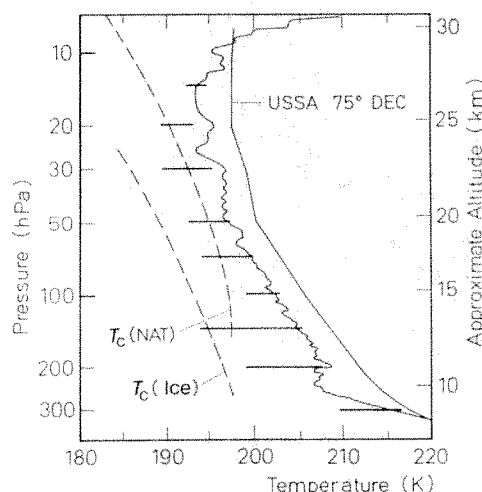


FIG. 2 Temperature profile measured on our balloon flight B88-2. For comparison we also show the US Standard Atmosphere zonally averaged temperatures for  $75^\circ\text{N}$  in December during cold conditions, and ranges for minimum temperatures measured in January and February over northern Sweden in 1982, 1983 and 1984 (horizontal bars). Also shown are model profiles for condensation temperatures for the NAT and  $\text{H}_2\text{O-ice}$  systems, calculated using our measured nitric acid abundance and a typical water vapour abundance of 5 p.p.m.v. (dashed curves).

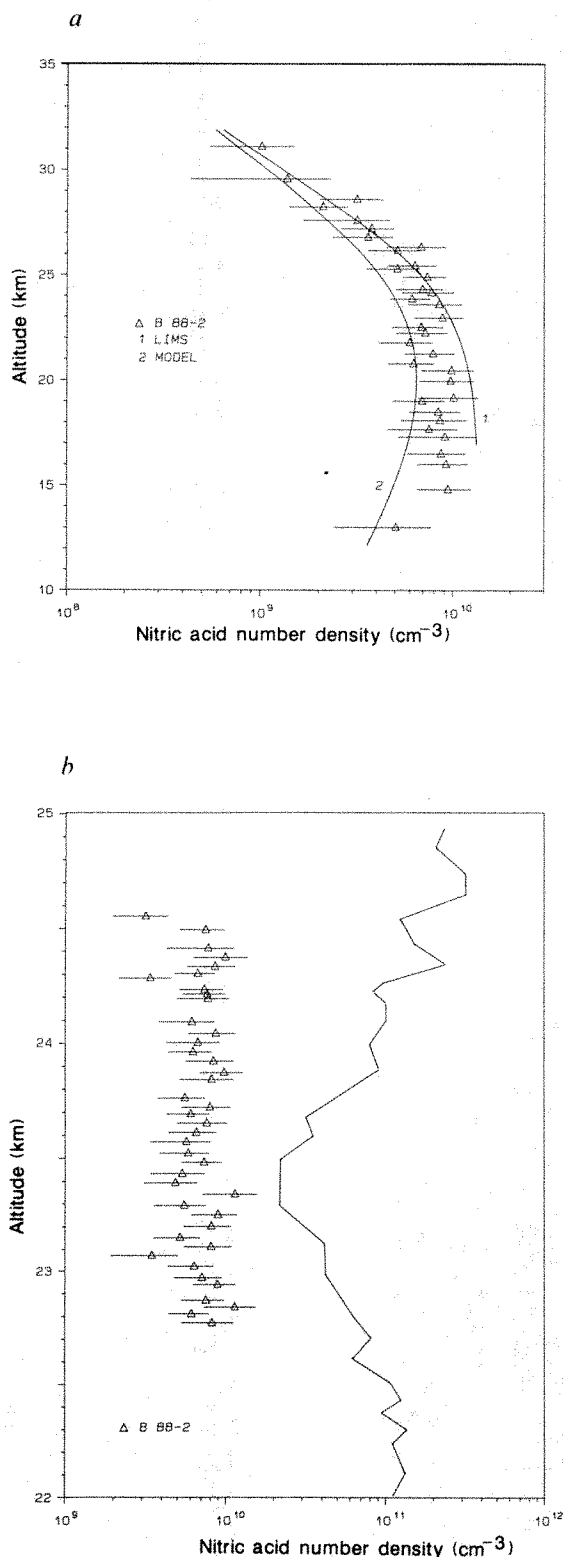


FIG. 3 *a*, Altitude distribution of concentrations of gaseous nitric acid measured by ACIMS on flight B88-2 (1 February 1988) in the cold Arctic winter vortex (the altitude is a geometric altitude measured by radar). For comparison, zonally averaged LIMS data<sup>9,10</sup> for December at 70° N are also given (curve 1). Comparison is also made with theoretical model calculations<sup>19,20</sup> for December at 70° N (in which heterogeneous formation of HNO<sub>3</sub> is neglected) (curve 2). *b*, Portion of the measured gaseous HNO<sub>3</sub> concentration profile shown in *a* but reprocessed to give the maximum possible height resolution. For comparison, model equilibrium saturation concentrations for HNO<sub>3</sub> over NAT are also shown, assuming a typical H<sub>2</sub>O vapour mixing ratio of 5 p.p.m.v. and using the measured atmospheric temperature profile (B88-2).

height distribution of  $T_c(\text{NAT})$  is shown in Fig. 2, along with  $T_c(\text{H}_2\text{O-ice})$ . We note, however, that water vapour abundance is a critical factor. Assuming a water vapour abundance of only 2.5 p.p.m.v.,  $T_c(\text{NAT})$  becomes lower by about 3 K; with a value of 7.5 p.p.m.v.,  $T_c(\text{NAT})$  increases by  $\sim 1$  K (ref. 8). Hence, local and temporal variations of water vapour would probably induce large local and temporal variations in  $T_c(\text{NAT})$  (of up to 4 K). Therefore, the  $T_c$  values for both NAT and pure water-ice (shown in Fig. 2) probably represent only approximate figures. The general decrease of  $T_c(\text{NAT})$  with increasing altitude is due to the decrease in concentrations of both HNO<sub>3</sub> and H<sub>2</sub>O. The steeper decrease above  $\sim 25$  km reflects the steep decrease of the HNO<sub>3</sub> concentration at these heights. The smaller gradient of the  $T_c(\text{NAT})$  curve below 15 km reflects the decrease in nitric acid concentration with decreasing height. The  $T_c(\text{NAT})$  curve becomes locally similar to our measured temperature profile at altitudes between  $\sim 18$  and 23 km, with  $\Delta T = T - T_c(\text{NAT})$  becoming locally as small as  $\sim 2$  K at around 23 and 20 km. Figure 2 also shows that zonally averaged temperatures<sup>26</sup> in December at a latitude of 75° N are markedly higher than  $T_c(\text{NAT})$ . However, minimum temperatures (shown in Fig. 2 for selected pressure levels) measured at the radiosonde station of Sodankylä (230 km east of Esrange) in January and February of 1982, 1983 and 1984 become distinctly lower than  $T_c(\text{NAT})$  at altitudes between about 17 km and 24 km and around 13 km. Around the latter height, minimum temperatures become even slightly lower than  $T_c(\text{H}_2\text{O-ice})$ . Hence, conditions for NAT aerosol formation seems to be favourable in two layers, at 17–24 km and  $\sim 13$  km, whereas H<sub>2</sub>O-ice aerosol formation seems to be possible only in the lower layer. It must be kept in mind, however, that these conclusions refer to only a single station (Sodankylä) and a period of only three years (1982, 1983 and 1984). The values of  $T_c(\text{NAT})$  and  $T_c(\text{H}_2\text{O-ice})$  at 23 km are shown in Fig. 1. Evidently, at the core of the vortex  $T$  became markedly lower than  $T_c(\text{NAT})$  from day 16 to day 64 (a period of seven weeks). In contrast, at Esrange  $T$  became lower than  $T_c(\text{NAT})$  at the 30 hPa-pressure level only during a single short period from around day 47 to day 51. With a model  $T_c(\text{NAT})$  value of 193 K at the 30-hPa level, the area where NAT aerosol formation may have occurred on 1 February 1988 is relatively large, approximately the size of Greenland.

In conclusion, it seems that the upper limit to  $T_c(\text{NAT})$  set by our present data is consistent with model predictions based on our nitric acid measurements, recent laboratory data<sup>8</sup> on macroscopic NAT mixtures and typical stratospheric abundances for water. The low  $T_c$  values mean that NAT aerosol formation may occur in the 'core' region of Arctic vortex but seems to be rare in outer regions of the vortex, at the latitude of Kiruna for example. Hence, the spatial and temporal distribution of NAT aerosol in the Arctic seems to be rather limited in extent compared with the Antarctic stratosphere, where average winter temperatures are about 5–10 K lower. However, lower temperatures may also reduce the probability of NAT formation by inducing heterogeneous condensation of 'pure' water vapour on NAT aerosols followed by rapid gravitational settling of relatively large water-ice particles containing NAT cores. Both nitric acid and water may thereby become depleted in the coldest layer, which would reduce the probability of subsequent NAT aerosol formation at these heights. Besides the larger dynamical activity, the higher temperatures of the Arctic vortex may therefore be a major reason for the absence of pronounced ozone depletion in the Arctic. However, a cooling of the winter polar Arctic vortex by only a few degrees may be sufficient to make NAT aerosol formation much more widespread in the Arctic. Such a cooling could be induced in the future by the rising content of CO<sub>2</sub> and other infrared active gases in the atmosphere, which cools the stratosphere by emission of infrared radiation. Recent climate models<sup>27</sup> predict a cooling of the Arctic vortex in winter by up to  $\sim 6$  K. Changes of the stratospheric water vapour abundance, which could result from changes of tropical

tropopause temperatures associated with a troposphere greenhouse effect, would also be important. □

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## Increased particle flux to the deep ocean related to monsoons

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**MONSOONS** cause seasonal reversals in the surface circulation of the northern Indian Ocean<sup>1</sup>. In the Arabian Sea this results in the upwelling of nutrient-rich water along the coasts<sup>2-4</sup>, making it one of the highly productive regions of the world's oceans. To assess the impact of monsoon-driven processes on the downward particle flux variations in the open ocean we deployed three moored arrays consisting of six time-series sediment traps at selected locations in the western, central and eastern parts of the deep northern Arabian Sea. Strong seasonality was recorded in particle flux at all three sites with peaks during the south-west and north-east monsoons. High primary productivity during the monsoons resulting from wind-induced mixed-layer deepening and the associated nutrient injection to the euphotic zone appeared to be the main factor controlling the observed particle flux pattern. These findings may shed light on CO<sub>2</sub> uptake during glaciation when wind speeds were higher.

Three mooring systems, each consisting of two time-series sediment traps—one 1,000 m below the sea surface and the other 1,000 m above the sea bottom—were deployed at three

sites in the western, central and eastern Arabian Sea (Fig. 1). The collecting cups were poisoned with mercuric chloride before deployment. The sediment traps were programmed to measure the flux of sinking particles at intervals of 12 to 13 days each over a duration of six months per deployment. They have been recovered and redeployed three times since May 1986 using the research vessels RV *Sonne* and the ORV *Sagar Kanya*. Here we report the results obtained during the first year of deployment.

On recovery of the traps, the samples were wet-sieved and split using a precision rotary splitter. One quarter of the <1 mm size fraction was filtered through pre-weighed Nuclepore filters (0.5 µm) and dried at 40 °C. This fraction was used for flux calculations and for analysis of first order parameters such as carbonate, opal, lithogenic, organic carbon, and nitrogen<sup>6,7</sup>.

At all three sites the flux of sinking particles showed seasonality which is strongly related to the monsoons with higher fluxes during the south-west (June to September) and north-east (December to February) monsoons (Fig. 2). Individual fluxes differed by more than two orders of magnitude. Differences between maximum and minimum fluxes during these two periods were highest at the western and eastern sites; these are characterized by their proximity to the upwelling centres along the Somali and the Arabian coasts and the Indian continental shelf, respectively. The similarity in flux patterns not only at sites close to the upwelling centres, but also at the site in the central Arabian sea, indicates that factors other than coastal upwelling might also be responsible for the higher particle fluxes. The close relationship observed between particle fluxes and wind speeds at the three sites provides a clue (Fig. 3). Higher wind speeds lead to a deepening of the mixed layer and the introduction of nutrient-rich subsurface waters into the euphotic zone<sup>8,9</sup>. Mixed-layer deepening from 30 to 40 m during the pre-monsoon period to more than 100 m during the south-west monsoon have been reported<sup>10</sup>. The wind-induced nutrient pumping into the euphotic zone is especially effective in the Arabian Sea as subsurface waters are extremely rich in nutrients<sup>11</sup>. This results in high primary production during both monsoons<sup>12-14</sup> and this high 'new production' is reflected in high particle fluxes to the deep sea. An exception to this is the eastern Arabian Sea during the north-east monsoon. Here particle fluxes are extremely low, although wind speeds are only slightly lower than at the other stations. This could be due to the absence of regular winter blooms caused by the inflow of low-salinity water from the eastern tropical Indian Ocean, which leads to stronger stratification and to lower productivity<sup>13</sup>.

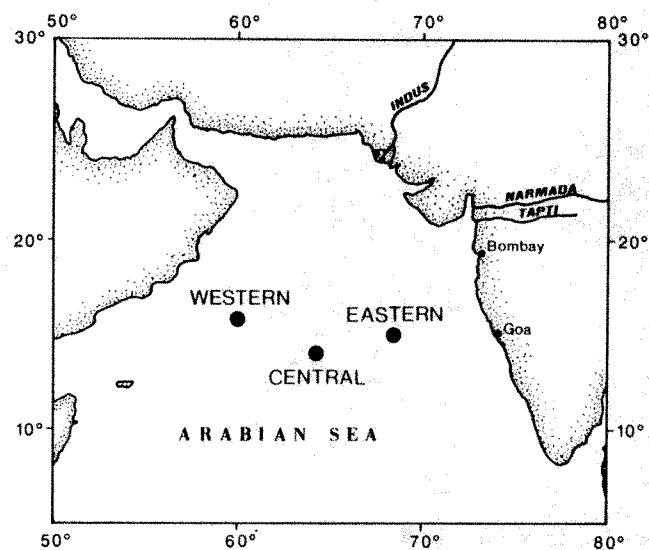
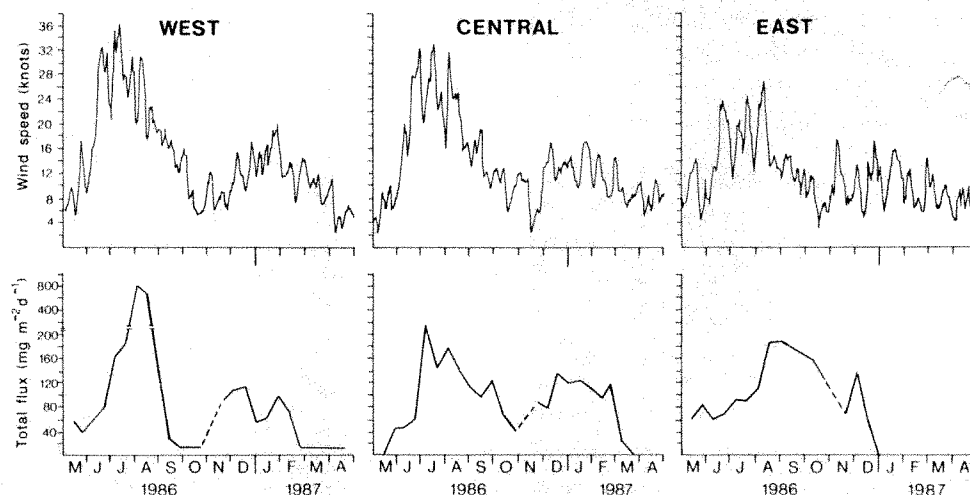


FIG. 1 Locations of mooring systems in the western, central and eastern Arabian Sea. The distance of the moorings from the nearest coast are 520, 930 and 430 km for the eastern, central and western trap, respectively.



FIG. 2 Seasonal variations of particle fluxes to the deep Arabian Sea (bottom) from time-series sediment trap deployments (sampling intervals 12–13 days) at the western, central and eastern Arabian Sea (trap depths: 3,020 m, 2,900 m and 2,770 m, respectively), and wind-speed data during the period of deployment (top) compiled from ship observations (Indian Daily Weather Report, Pune, India) within a radius of  $2^\circ$  around the three sediment trap locations. Wind-speed data were smoothed with a 5-point moving average. Peak fluxes occurring during the SW (June–September) and NE (December–February) monsoons show close similarity to wind-speed patterns and are related to high primary production resulting from wind-induced mixed layer deepening and associated nutrient injection in the euphotic zone<sup>8,9</sup>, and is best established in the central Arabian Sea (see Fig. 3). The peak fluxes in the western Arabian Sea during July–August and between August–October in the eastern Arabian Sea also have components derived from nutrient advection<sup>16</sup> and lateral transport of material from the coastal



upwelling centres. In the eastern Arabian Sea strong stratification results from the inflow of low-salinity water from the eastern tropical Indian Ocean during the NE monsoon leads to low productivity<sup>13</sup> reflected in low particle fluxes.

Further information on factors controlling the observed fluxes comes from the component fluxes (Table 1). Carbonates dominate the component fluxes and are present mainly in the form of coccolithophorids with a certain amount of foraminifera. Organic carbon and nitrogen fluxes are similar to those reported from other oceanic areas<sup>15</sup>. Major differences between sites were observed in the opal and lithogenic fluxes.

In the central and eastern Arabian Sea sites, opal accounts for 12% and 13% of the total flux. In the western Arabian Sea, opal contributed up to 22% of the total annual flux and accounted for as much as 40% during the peak fluxes in July and August, mostly in the form of the diatom *Rhizosolenia*, a major upwelling species. During the summer monsoon of 1986, waters from the Somali-Arabian upwelling centres have been shown to be advected up to  $60^\circ$  E (ref. 16). Peak fluxes at this site located more than 400 km from the continental margin thus have a component derived from nutrient-rich upwelled water transported offshore.

High lithogenic fluxes are associated with the south-west monsoon (June to September) and their contribution to total fluxes increases from the western (9%) to the eastern (25%)

Arabian Sea site (Table 1). Eolian input to the Arabian Sea is restricted by the Somali Jet to the northwestern parts, and may constitute an important source of lithogenic particles at the western Arabian Sea site (ref. 17; F. Sirocco and M. Sarnthein, manuscript in preparation). In the northeastern and central parts of the Arabian Sea the chief suppliers of lithogenic material are the rivers Indus, Narmada and Tapi. More than 80% of their annual suspended-matter discharge occurs during the south-west monsoon<sup>18</sup>. The simultaneous prevalence of clockwise surface currents in the Arabian Sea can divert lithogenic material to the eastern Arabian Sea, contributing to its relative enrichment. Sediment-trap studies in the outer shelf off Goa, India, have shown Indus-derived sediment to be a significant component during this period<sup>19</sup>.

Our results show that the particle fluxes out of the surface layers in the Arabian Sea are primarily linked to changes in the prevailing meteorological and hydrographical conditions through biological processes occurring in the surface layers. Higher wind speeds promote biological productivity by (1) enhancing the introduction of essential trace elements associated with atmospheric dust fallout (for instance, iron<sup>20</sup>), and (2) the

TABLE 1 Component fluxes of the fraction  $<1$  mm to the deep Arabian Sea. The data are given for the SW (June–September), NE (December–February) monsoons and for the periods in between

Site	Components <1 mm	NE-SW		SW Monsoon		SW-NE		NE monsoon		Total	
		$\text{g m}^{-2}$	%	$\text{g m}^{-2}$	%	$\text{g m}^{-2}$	%	$\text{g m}^{-2}$	%	$\text{g m}^{-2}$	%
Western Arabian Sea	carbonate	1.17	54.2	12.10	51.4	2.13	73.2	3.60	68.1	19.00	56.00
	opal	0.12	5.6	6.08	25.8	0.30	10.3	0.78	14.7	7.28	21.5
	lithogenic	0.11	5.1	2.10	8.9	0.28	9.6	0.15	2.8	2.64	7.8
	$\text{C}_{\text{org}}$	0.16	7.4	1.18	5.0	0.14	4.8	0.32	6.0	1.80	5.3
	N	0.02	0.9	0.14	0.6	0.02	0.7	0.04	0.8	0.22	0.6
	Total flux	2.16	6.4	23.56	69.5	2.91	8.6	5.29	15.6	33.92	
Central Arabian Sea	carbonate	0.49	57.6	9.31	66.4	2.15	66.8	5.55	66.4	17.50	66.2
	opal	0.06	7.1	1.51	10.8	0.35	10.9	1.16	13.9	3.08	11.6
	lithogenic	0.07	8.2	1.84	13.1	0.35	10.9	0.79	9.5	3.05	11.5
	$\text{C}_{\text{org}}$	0.06	7.1	0.74	5.3	0.20	6.2	0.53	6.3	1.53	5.8
	N	0.01	1.2	0.09	0.6	0.02	0.6	0.07	0.8	0.19	0.7
	Total flux	0.85	3.2	14.02	53.0	3.22	12.2	8.36	31.6	26.45	
Eastern Arabian Sea	carbonate	0.80	43.2	7.27	49.5	3.40	54.4	0.41	51.3	11.88	50.4
	opal	0.30	16.2	1.81	12.3	0.79	12.6	0.23	28.8	3.13	13.3
	lithogenic	0.42	22.7	3.60	24.5	1.35	21.6	0.03	3.8	5.40	22.9
	$\text{C}_{\text{org}}$	0.18	9.7	1.00	6.8	0.33	5.3	0.05	6.3	1.56	6.6
	N	0.02	1.1	0.10	0.7	0.04	0.6	0.01	1.2	0.17	0.7
	Total flux	1.85	7.8	14.68	62.3	6.25	26.5	0.80	3.4	23.58	

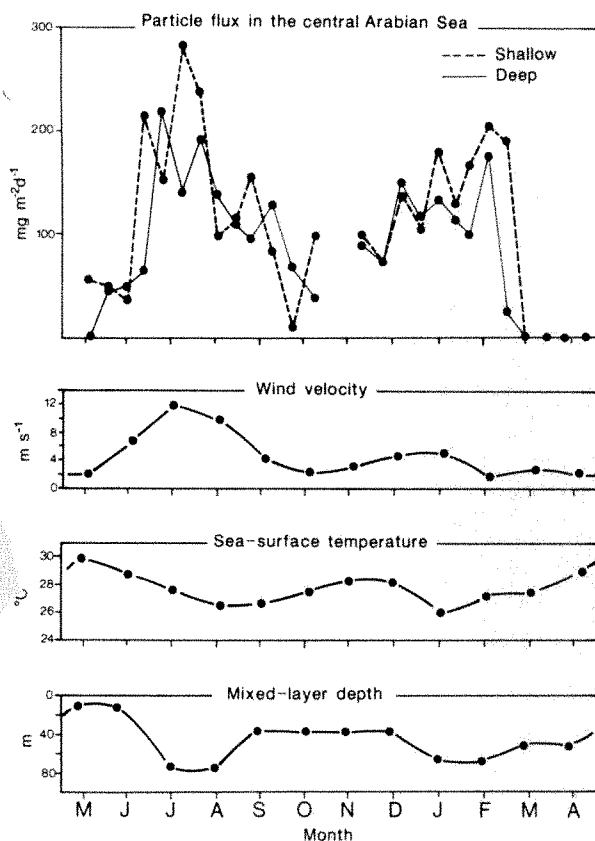


FIG. 3 Time-series (sampling intervals 12–13 days) of particle flux from two depths (shallow, 780 m; deep, 2,900 m) in the central Arabian Sea, and the general information on wind velocity<sup>23</sup>, sea-surface temperature<sup>2</sup> and mixed layer depth<sup>1</sup>. Low sea-surface temperatures and a deeper mixed layer during these periods show that wind-induced mixing occurs which brings nutrient-rich water to the surface. The resultant high biological productivity leads to the observed flux pattern.

injection of new nutrients from deeper layers to the euphotic zone. There have been times in the recent geological past when wind speeds were higher than today's by a factor of 1.3 to 1.6, and the atmosphere had higher dust contents, for example during the glacial periods<sup>21</sup>, which must have led to an increase in global ocean productivity. The subsequent removal of this new production to the deep ocean as deduced from our study could have contributed in part to low atmospheric CO<sub>2</sub> contents during the glacial periods<sup>22</sup>. □

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## Barium content of benthic foraminifera controlled by bottom-water composition

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THE carbon isotope ratio ( $\delta^{13}\text{C}$ ) and cadmium content (Cd/Ca) of benthic foraminifera shells have been used to reconstruct deep-water circulation patterns of the glacial oceans<sup>1–7</sup>. These tracers co-vary with phosphorus in the modern ocean because they are nearly quantitatively regenerated from sinking biological debris in the upper water column. Hence they can be used to reconstruct the distribution of labile nutrients in glacial water masses. Independent constraints on glacial deep-ocean circulation patterns could be provided by a tracer of the distribution of silica and alkalinity, the deeply regenerated constituents of planktonic hard parts. Barium shares key aspects of its behaviour with these refractory nutrients because it is removed from solution in surface waters and incorporated into sinking particles which slowly dissolve deep in the water column and in the sediments<sup>8</sup>. The fractionation of Ba between deep-water masses of the major ocean basins is largely controlled by thermohaline circulation patterns, so Ba conforms to different boundary conditions from Cd and  $\delta^{13}\text{C}$ . As Ba substitutes into trigonal carbonates<sup>9</sup>, it is a potential palaeoceanographic tracer if the Ba content of foraminifera shells reflects ambient dissolved Ba concentrations. Here we present data from Recent core-top benthic foraminifera which indicate that the Ba content of some recent calcitic benthic foraminifera does co-vary with bottom-water Ba.

The distribution of barium, alkalinity and silica in the world's oceans was mapped extensively during the GEOSECS

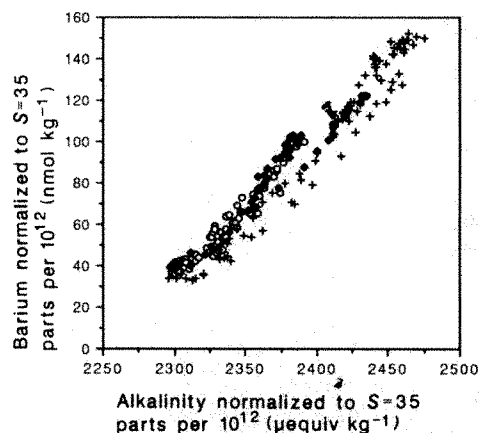


FIG. 1 Paired Ba and alkalinity data from ocean water samples analysed for the GEOSECS program<sup>10–13</sup>. Alkalinity and barium are normalized to a constant salinity of 35‰. Included are: Atlantic Ocean stations 29, 82 and 111 (circles); Pacific Ocean stations 204, 226, 312 and 322 (crosses); and Indian Ocean stations 429 and 452 (diamonds).

program<sup>10-13</sup>. Figure 1 illustrates the similarity between Ba and alkalinity over nine widely spaced GEOSECS sites. Although the cycling of these two tracers is dominated by different mechanisms<sup>14,15</sup>, the overprint of thermohaline circulation on two dissolved constituents sharing similar sites of uptake and regeneration results in strong covariance throughout the world's oceans. Thus the distribution of Ba in glacial oceans can provide direct insight into the distribution of refractory nutrients such as alkalinity.

To calibrate the response of benthic foraminifera shells to ambient Ba concentrations we have measured Ba in foraminifera recovered from Recent core tops lying below bottom waters whose Ba concentrations can be estimated from nearby GEOSECS stations. Foraminifera were hand-picked from disaggregated sediment samples and cleaned with a series of physical and chemical steps designed to remove any extraneous Ba not substituted into the lattice of the foraminiferal  $\text{CaCO}_3$ . These steps begin with the foraminiferal Cd cleaning method<sup>6</sup>, followed by dissolution of associated sedimentary barite ( $\text{BaSO}_4$ ) using alkaline diethylenetriamine-pentaacetic acid (DTPA)<sup>16</sup>. The typical sample mass before cleaning is 0.6 mg (10-30 individuals). Barium concentrations are quantified by isotope-dilution flow-injection analysis on an inductively coupled

plasma-mass spectrometer (ICP-MS). Samples are spiked with  $^{135}\text{Ba}$  and the  $^{135}\text{Ba}/^{138}\text{Ba}$  ratio is measured subsequently. Accuracy is based on calibration of isotope-dilution determinations to a gravimetric standard. The detection limit of the flow-injection method is less than  $0.2 \text{ nmol l}^{-1}$  for a 200- $\mu\text{l}$  injection volume (5 pg Ba). Signal-to-noise ratios of samples were generally greater than 50:1. Calcium is quantified by flame atomic absorption. The inter-run reproducibility for 39 analyses of a consistency standard containing  $9.6 \text{ nmol l}^{-1}$  Ba and  $4.1 \text{ mmol l}^{-1}$  Ca ( $\text{Ba/Ca} = 2.3 \mu\text{mol mol}^{-1}$ ) was 2.6% for Ba, 1.5% for Ca and 3.1% for the Ba/Ca ratio. Although contamination during sample preparation and handling is negligible, occasional individual analyses are unreliable because of insufficient cleaning.

The Ba/Ca ratio of the three species studied (Fig. 2a, b and c; Table 1) increases linearly with increasing concentration in the bottom water. The scatter in the plots exceeds analytical precision and uncertainties in estimating Ba from nearby stations. This scatter may result either from bioturbation, which can mix older individuals (which may have lived at times when bottom-water Ba concentrations were different) into the core top, or from a limit on the reliability of certain species of foraminifera as recorders of ambient Ba concentrations. We

TABLE 1 Ba/Ca ratios in benthic foraminifera from Recent core tops

Core	Sample depth (cm)	Latitude	Longitude	Water depth (m)	Estimated bottom-water Ba ( $\text{nmol kg}^{-1}$ )	Ba/Ca ( $\mu\text{mol mol}^{-1}$ )		
						<i>C. wuellerstorfi</i>	<i>C. kullenbergi</i>	<i>Uvigerina</i> spp.
AI154 5PG	2-4	07°25' S	89°10' W	4,165	133	4.18		4.16
AI107 65GGC	4-7	32°02' S	36°11' W	2,795	64	2.15		
AI107 67GGC	0-2	31°55' S	36°12' W	2,587	63	$1.89 \pm 0.06$ ( $n=2$ )		
AI107 69GGC	5-7	31°40' S	36°01' W	2,158	66			2.26
AI107 70GGC	2-4	31°36' S	35°59' W	2,079	66	2.20		2.04
AI107 71GGC	0-2	31°31' S	35°56' W	1,887	68	2.24		2.00
CHN82 1PC	2-4	36°06' N	07°10' S	830	47			2.18
CHN82 3PC	13-16	41°38' N	27°20' W	2,525	51	1.77		
CHN82 4PC*	0-2	41°43' N	32°51' W	3,427	56	2.44	$2.26 \pm 0.01$ ( $n=2$ )	
CHN82 9PC	7-10	41°51' N	26°27' W	2,830	52	1.85		
CHN82 11PC*	1-6	42°23' N	31°48' W	3,209	55	2.25	$2.18 \pm 0.06$ ( $n=2$ )	
CHN82 15PC	3-5	43°14' N	28°08' W	2,155	50	$1.98 \pm 0.02$ ( $n=3$ )		$2.63 \pm 0.16$ ( $n=2$ )
CHN82 20PG*	4-7	43°30' N	29°52' W	3,070	53	1.82	$2.52 \pm 0.04$ ( $n=2$ )	
CHN82 21PG	4-8	43°17' N	29°50' W	2,103	49	1.92	2.67	
EN66 10GGC	1-2	06°39' N	21°54' W	3,527	74	2.58	2.28	
EN66 16GGC	1-3	05°28' N	21°08' W	3,152	73	2.79	2.87	
EN66 21GGC*	3-12	04°14' N	20°38' W	3,995	75	2.75	2.35	
EN66 26GGC	1-3	03°05' N	20°01' W	4,745	77	2.86		
EN66 32GGC*	0-3	02°28' N	19°44' W	5,003	77	3.00		
EN66 36GGC	2-3	04°19' N	20°13' W	4,270	75	2.45		
EN66 38GGC	2-4	04°55' N	20°30' W	2,931	71	2.31	2.39	2.56
EN66 44GGC	1-2	05°16' N	21°43' W	3,428	73	2.89	2.72	
IOS82 PCS01	2-4	42°23' N	23°31' W	3,540	61		2.54	
KNR64 5PG	6-8	16°32' N	74°48' W	3,047	56	2.26	2.42	
KNR73 3PC*	5-6	00°22' S	106°11' W	3,606	134	4.95		
OC173 G	4-6	31°54' N	64°18' W	4,469	65	$2.16 \pm 0.10$ ( $n=3$ )		
PS21295-4*	1-2	78°00' N	02°25' E	3,112	46	2.03		
RC11-120*	5-7	43°31' S	79°52' E	3,135	91	3.41		
TR163-14	3-5	05°41' N	87°14' W	2,365	132	4.12		
TR163-27	2-3	02°15' S	86°35' W	3,180	135			4.66
TR163-28	1-2	29°19' S	86°14' W	3,200	135			4.09
TR163-31B*	1-11	03°57' S	85°58' W	3,210	133	$4.41 \pm 0.24$ ( $n=3$ )		$4.02 \pm 0.47$
TR163-32	2-4	02°29' S	82°59' W	2,890	134		5.04	$3.93 \pm 0.15$ ( $n=2$ )
V18-68*	10-12	54°33' S	77°51' W	3,972	110	3.43		3.94
V22-174*	9-11	10°04' S	12°49' W	2,630	76			1.94
V22-197*	14-17	14°10' N	18°35' W	3,167	72	3.10		2.80
V22-198	4-6	14°35' N	19°40' W	1,082	62		2.52	
V24-109	5-8	00°26' N	158°48' E	2,367	136	3.76		
V27-60	5-8	72°11' N	08°34' E	2,525	49	2.11		
V27-86*	5-8	66°36' N	01°07' E	2,900	51	1.90		
V28-56*	3-4	68°02' N	06°07' W	2,941	51	1.97		
V28-304	6-9	28°32' N	134°08' E	2,942	139	3.69		
V32-159	15-17	48°40' N	147°24' E	1,235	109			2.94

\* Cores with >15 cm Holocene sediment



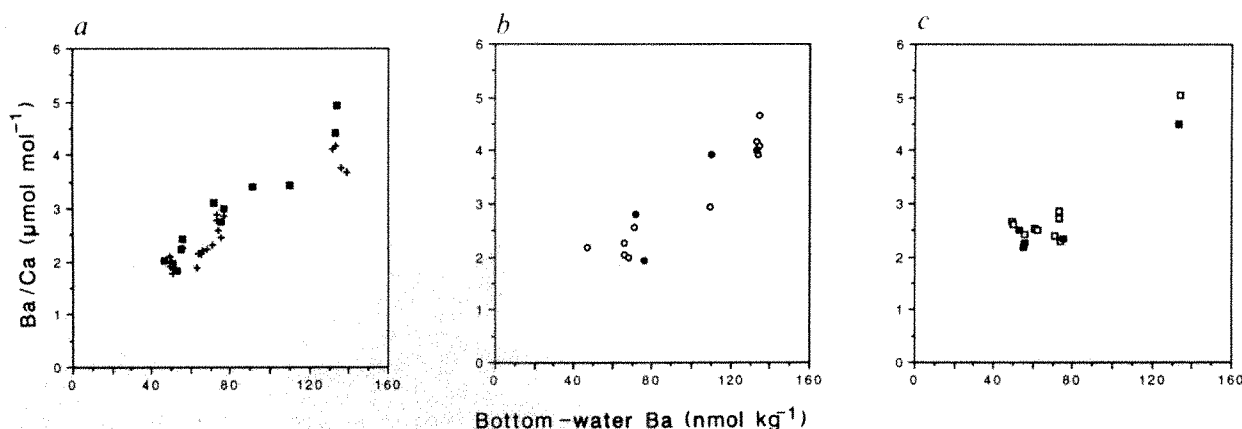


FIG. 2 Ba/Ca in recent benthic foraminifera plotted against estimated bottom-water barium concentrations. Samples from cores with >15 cm of

Holocene sediment are indicated by darkened symbols. a, *C. wuellerstorfi*; b, *Uvigerina spp.*; c, *C. kullenbergi*.

believe the first factor to be a primary cause of scatter for *C. wuellerstorfi* (Fig. 2a) because the most deviant points (low Ba/Ca ratios for high-Ba Pacific points) are from cores with lower sedimentation rates. Because data for the other two species are more limited, we can not clearly identify the cause of the offsets. Of the three species, *C. wuellerstorfi* shows the least scatter. However, we cannot conclude that this species is necessarily a more reliable recorder of bottom-water Ba because bioturbation combined with temporal abundance variations can bias core tops such that the mean age of each species present might not be the same. Because *Uvigerina spp.* is generally low in abundance during interglacials, *Uvigerina spp.* recovered from core tops are probably less likely to be representative of recent conditions than are *C. wuellerstorfi*.

We average the data in Fig. 2 to calculate an effective distribution coefficient  $D = (\text{Ba/Ca})_{\text{foraminifera}} / (\text{Ba/Ca})_{\text{sea water}}$ . For the fractionation of Ba in the calcite of the three benthic species,  $D = 0.37 \pm 0.06$ . In the present data set there is no evidence for significant differences in individual  $D$  values for each species, although mean  $D$  values range from 0.34 for *Uvigerina spp.* and 0.37 for *C. wuellerstorfi* to 0.41 for *C. kullenbergi*. The uncertainty in  $D$  for each species can be improved by further analyses from high-sedimentation-rate cores, ultimately allowing us to determine whether different benthic species have different effective distribution coefficients.

The estimate of the foraminiferal distribution coefficient applies only to cold deep waters (temperature  $\sim 3^\circ\text{C}$ , pressure  $\sim 300$  bar). Inorganic-precipitation experiments performed at 20–25°C and 1 bar pressure generally yield distribution coefficients of the order of 0.1, although much higher values are observed during the first stages of precipitation or during rapid precipitation<sup>9</sup>. Observed foraminiferal  $D_s$  for chemically similar Sr is about a factor of four times higher than that observed for slow precipitation<sup>17–19</sup>. Measurements of Ba in planktonic foraminifera indicate an effective distribution coefficient of about 0.2 (ref. 20), although the restricted range of Ba concentrations in surface waters limits validation of the direct response of planktonic foraminifera to changes in surface-water Ba concentrations. That this value is significantly different from that for benthic foraminifera suggests that variables such as temperature, pressure and biological factors may play a role in determining  $D$ .

Thus it will be possible to use fossil shells from deep-sea cores to reconstruct the distribution of Ba in the bottom waters of past oceans. A preliminary examination of cores from the North Atlantic indicates that benthic foraminifera recovered from glacial sections have up to 50% higher Ba/Ca ratios than are observed for the Holocene individuals. Higher glacial Ba in the deep Atlantic is an indication of reduced flushing of the Atlantic by nutrient-depleted waters during glacial periods. □

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## Stress dependence of the mechanism of the olivine–spinel transformation

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OLIVINE,  $\alpha\text{-(Mg, Fe)}_2\text{SiO}_4$ , is the most abundant phase in the Earth's upper mantle. It transforms to high-density polymorphs ( $\gamma$ , with the spinel structure and  $\beta$ , with a modified spinel structure) under pressure and temperature conditions appropriate to explain the discontinuity in seismic velocities that defines the base of the upper mantle at 400 km depth; the transformation is generally believed to be responsible for that discontinuity<sup>1</sup>. Knowledge of the mechanism and kinetics of the transformation is important for understanding certain aspects of mantle dynamics, and extensive experimental and theoretical work has been conducted on both natural olivine and several analogous chemical systems. Previously, conflicting results have suggested a reconstructive transformation mechanism<sup>2–10</sup>, a martensitic mechanism<sup>11,12</sup> and a mechanism that is not entirely consistent with either<sup>13–16</sup>. The conflicts cannot be explained as different mechanisms operating in different systems<sup>17</sup>. Here we show that the transformation can proceed by two



different mechanisms, depending on the level of nonhydrostatic stress; under hydrostatic pressure or low stresses (at sufficiently high temperatures), nucleation occurs by an incoherent, diffusion-dependent, intercrystalline mechanism proposed by Sung and Burns<sup>2,3</sup> and first demonstrated by Vaughan *et al.*<sup>4</sup> (Fig. 1), whereas under high stresses, nucleation of the high-pressure ( $\gamma$ ) polymorph (spinel) occurs by a coherent, shear-induced, intracrystalline mechanism predicted by Poirier<sup>18</sup>. Thus we are able to reconcile the previously conflicting results, and consideration of the stress levels likely in natural situations leads us to conclude that both mechanisms are geologically relevant.

As part of a continuing study of the effect of stress on the olivine-spinel transformation<sup>19-21</sup>, we deformed specimens of a synthetic  $\text{Mg}_2\text{GeO}_4$  olivine polycrystal (an analogue of the mantle material) at a strain rate of  $10^{-4} \text{ s}^{-1}$ , a temperature of 900 K and pressures of 1.1–1.4 GPa in a modified Griggs apparatus<sup>22</sup>. These conditions are in the spinel stability field, a minimum of 400 K below the equilibrium phase boundary. The specimens are extremely strong, displaying a flow stress ( $\sigma_1$ – $\sigma_3$ ) of 1.9 GPa or more in all cases (Table 1).

Optical microscopy of sections of specimens quenched immediately after deformation showed extremely inhomogeneous deformation within individual olivine crystals, but martensitic (stress-induced) lamellae of the high-pressure polymorph were not found. Examination by transmission electron microscopy (TEM), however, revealed (Fig. 2) thin (1–8 nm) lamellae parallel to (100). (To avoid confusion, we index  $\text{Mg}_2\text{GeO}_4$  olivine according to the unit cell for silicate olivine, in which  $b > c > a$ .) Selected-area electron diffraction (SAED) patterns showed pronounced streaking parallel to the (100) reciprocal lattice direction, but no diffraction spots attributable to the spinel phase were found (Fig. 2, insert), even at specimen strains approaching 35% shortening. The streaking in the diffraction patterns is attributable to the thin lamellae. X-ray diffraction analysis of powders prepared from these specimens also failed to show any spinel lines.

Deformed specimens annealed at 1200 K (at pressure) for longer than 15 min yielded optically visible, faceted spinel crystals; after 1 hour, spinel crystals as large as  $80 \mu\text{m}$  developed, the average diameter being  $50 \mu\text{m}$  (Fig. 3a). The spinel grains nucleated within the olivine crystals, in contrast to spinels grown at higher temperature under hydrostatic or low-stress conditions, which nucleate exclusively at grain boundaries. The interfacial angles between crystal facets indicate that the spinel habit is {111}; growth of several crystals until they interfere commonly leads to irregular shapes, but the {111} facets remain visible (Fig. 3a). Within a single olivine crystal, one {111} facet of each spinel crystal is parallel to one facet of the other spinels and to (100) of the host olivine. Examination of annealed specimens by TEM (Fig. 3b) revealed coarser lamellae than those present in unannealed specimens, and also larger spinel crystals which had lost the lamellar habit. SAED patterns of regions containing coarser lamellae retained the streaking of the as-deformed material, but displayed extra spots characteristic of spinel. Indexing of the patterns showed that  $[100]_{\text{ol}} = [111]_{\text{sp}}$  and  $[001]_{\text{ol}} = [\bar{1}10]_{\text{sp}}$ . The larger crystal of Fig. 3b, although having lost its lamellar habit, retained its largest dimension parallel to



FIG. 1 High-voltage (1,000 kV) transmission electron micrograph of several elongated spinel ( $\gamma$ ) crystals growing into a single olivine ( $\alpha$ ) crystal (white). The variation of contrast and orientation of the planar and linear defects in the spinels demonstrates their widely differing orientations. Electron diffraction confirmed these differences<sup>4,9</sup>, and showed that none of the crystals exhibit the topotaxy predicted by Poirier<sup>18</sup>. Scale bar,  $1 \mu\text{m}$ .

(100)<sub>ol</sub> and exhibited the same topotaxy as the lamellae. By contrast, a specimen annealed under the same conditions but not previously deformed developed no lamellae and no spinel crystals. Our observation of loss of the lamellar habit as coarsening begins suggests that crystal growth occurs by normal phase-boundary migration, leading to euhedral crystals bounded by {111} faces.

The lamellae have all of the characteristics predicted by the martensitic mechanism of Poirier<sup>18</sup>, who constructed his model of the transformation mechanism by analogy with similar martensitic transformations in other systems. In those systems, softening of the appropriate elastic stiffness moduli is observed in the vicinity of the phase boundary. Accordingly, Poirier<sup>18</sup> predicted that the elastic stiffness coefficients  $C_{55}$  and  $C_{66}$  of olivine should show similar premonitory phenomena. A search for such shear-mode softening in  $\text{Fe}_2\text{SiO}_4$  was unsuccessful<sup>23</sup>. We propose that failure of  $C_{55}$  and  $C_{66}$  to soften is complementary to our observations, first that a very high stress is necessary for nucleation by the martensitic mechanism, and second that growth of the  $\gamma$  phase under hydrostatic pressure occurs by

TABLE 1 Experimental results

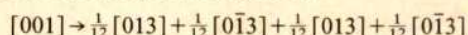
$T$ (K)	$P$ (GPa)	$\dot{\epsilon}$ ( $\text{s}^{-1}$ )	Maximum stress (GPa)	Total strain (%)	Length of 1,200-K anneal (min)	Spinel visible optically	Lamellae visible in TEM	Sample number
902	1.4	$1.4 \times 10^{-4}$	2.5	23	0	no	yes	GL294
897	1.3	$3.2 \times 10^{-4}$	1.9	34	2	no	yes	GL306
897	1.1	$2.4 \times 10^{-4}$	2.2	12	18	yes	yes	GL309
898	1.3	$3.4 \times 10^{-4}$	2.7	41	58	yes	yes	GL307
1,391	1.7	$2.4 \times 10^{-5}$	0.2	10	—	yes	no	GL264

$\dot{\epsilon}$  is strain rate.



normal phase-boundary migration rather than by invasion of the crystal by large numbers of partial dislocations.

These new microstructural results not only indicate that there are two separate mechanisms which operate under different conditions, but also provide the information necessary to reconcile the differences between previous studies<sup>17</sup>. In particular, our new results lead to an understanding of why low-temperature experiments using synchrotron radiation<sup>15,16</sup> showed lack of cation ordering in the early stages of the transformation, even though they indicated volume contraction simultaneous with oxygen restacking<sup>17</sup>. The answer is inherent in Poirier's model. Briefly, Poirier<sup>18</sup> proposed that under conditions appropriate for the transformation, olivine dislocations with a  $[001]$  Burgers' vector gliding on the  $(100)$  plane separate into four partial dislocations according to the reaction:



Implicit in this dislocation mechanism is the fact that a spinel layer produced by passage of the first partial dislocation will have an antiphase relationship with respect to a spinel layer produced by passage of the third partial dislocation (see also Hornstra<sup>24</sup>). Because the individual lamellae are thin and closely

spaced compared with the minimum dimension resolvable by X-ray diffraction, therefore within the same crystal, groups of lamellae with antiphase relationships will be summed by synchrotron radiation, giving rise to anomalously low apparent structure factors for reflections other than those from the oxygen sublattice. As individual crystal domains grow to dimensions resolvable by X-ray diffraction, so too will grow the intensities of the cation-related peaks. In other words, the synchrotron results have been interpreted correctly in that they require cation disorder on the scale of resolution of the technique ( $\sim 100$  nm), whereas in rejecting Poirier's mechanism, the implication is that cation disorder is required on the scale of Poirier's model ( $\sim 1$  nm).

These results also clarify a number of other confusions in the literature. Hamaya and Akimoto<sup>13,14</sup> grew large, euhedral  $\text{Ni}_2\text{SiO}_4$  spinels within olivine single crystals. Some of their

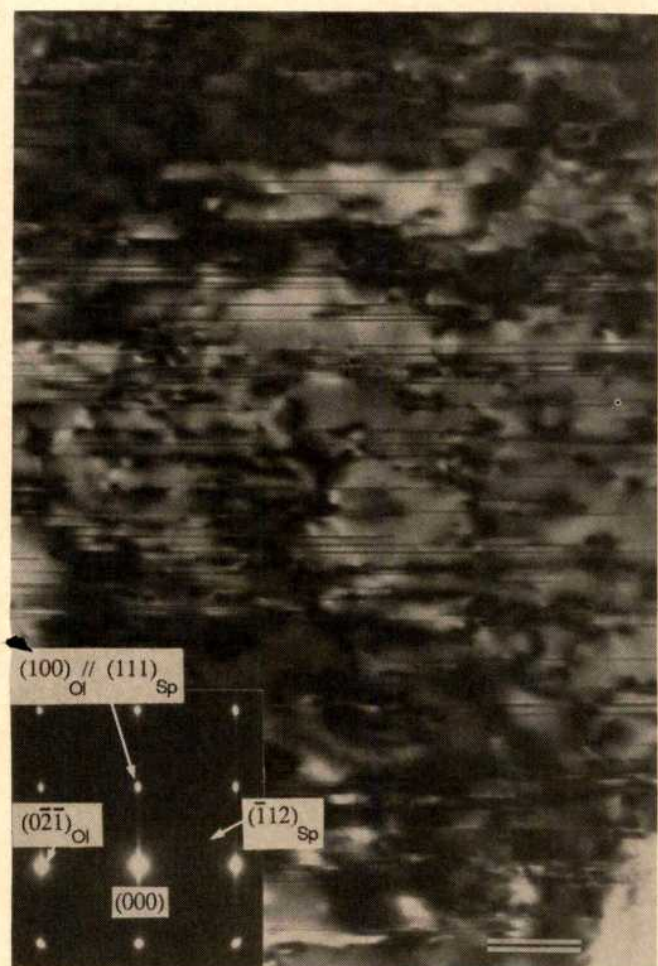


FIG. 2 Transmission electron micrograph and selected-area electron diffraction pattern, suggesting a martensitic nucleation mechanism for the  $\alpha \rightarrow \gamma$  phase transformation. The micrograph is viewed parallel to  $[0\bar{1}2]_{\text{Ol}}$ . Thin lamellar features are parallel to  $(100)$ . No structure of this type in olivine has previously been seen by the authors or reported in the literature, except for Fig. 1 of ref. 12. Some lamellae can be seen to end. Fuzzy black areas are unit dislocations in poor contrast. The high density of slip dislocations makes clear imaging of the lamellae difficult. The insert in the lower left corner is a selected-area electron diffraction pattern, showing streaking normal to  $(100)_{\text{Ol}}$ . Spots corresponding to spinel (e.g.  $[\bar{1}12]_{\text{Sp}}$ ) are not present (arrow). Scale bar,  $0.1 \mu\text{m}$ .



FIG. 3 Annealing at  $1,200$  K of specimens deformed at  $900$  K results in growth of large spinel crystals in the olivine matrix. *a*, Photomicrograph taken between crossed polarizers, showing growth of multiple spinel crystals (isotropic) within a single large olivine grain. The spinels have grown together in many cases, resulting in irregular masses, but each shows a single  $\{111\}$  face (indicated by arrowheads) parallel to that of the other spinels and parallel to the trace of  $(100)$  of olivine (E-W direction of micrograph). *b*, Transmission electron micrograph showing lamellae parallel to  $(100)_{\text{Ol}}$  and a larger, faceted spinel crystal which is elongated parallel to  $(100)_{\text{Ol}}$ , but which does not have a lamellar habit. The larger crystal facet (larger arrowhead) is a  $\{111\}_{\text{Sp}}$  face parallel to  $(100)_{\text{Ol}}$ . The other facets are other  $\{111\}$  planes (smaller arrowheads). Selected-area electron diffraction (SAED) of the lamellar region (insert, lower right) shows weak streaking perpendicular to  $(100)_{\text{Ol}}$  with prominent olivine spots and secondary spinel spots that confirm the topotaxy predicted by Poirier<sup>18</sup>. SAED of the larger spinel crystal (insert, lower left) shows that the position and relative intensity of the spots are identical to those of the spinel spots in the lamellar region, confirming that the larger crystal has exactly the same orientation as the lamellae and indicating that it grew from a lamellar nucleus. Scale bars:  $20 \mu\text{m}$  in panel *a*;  $0.1 \mu\text{m}$  in *b*.



crystals exhibited the topotaxy predicted by Poirier's model, but other crystals displayed the octahedral crystal habit and lacked the topotaxy, making interpretation difficult. In the light of our annealing results, we believe that the simplest explanation for their observations is that stress concentrations produced during pressurization in their apparatus led to production of martensitic nuclei in the earliest stages of their experiments and that coarsening of those nuclei by normal phase-boundary migration during the experiment produced their large, euhedral, crystals. At the temperatures of their experiments, incoherent nuclei were also produced, leading to the crystals that did not display topotaxy with the olivine.

The present results resolve the discrepancies in the literature concerning the mechanism of the transformation in static or quasi-static experiments. The results also imply that the nucleation mechanism should always be martensitic in shock experiments. Indeed, the extreme stresses in shock deformation might lead to complete transformation by the martensitic mechanism rather than the coarsening by normal phase-boundary migration reported here.

One must use caution, however, when extrapolating these results to natural environments, because none of the studies summarized above includes the complication of the  $\beta$  phase<sup>1,17</sup> (which does not exist for most of the analogue materials, including  $\text{Mg}_2\text{GeO}_4$ ). We believe, however, that under most circumstances extrapolation to natural environments should be straightforward. At high temperature and low stress,  $\alpha$  transforms to  $\gamma$  by incoherent nucleation and growth in all systems studied, and  $\gamma \rightarrow \alpha$  in  $\text{Mg}_2\text{GeO}_4$  (ref. 21) and  $\alpha \rightarrow \beta$  in  $\text{Co}_2\text{SiO}_4$  (ref. 25) also follow this mechanism. Therefore, in normal mantle environments, where the temperature is high and stresses are very low, the forward or reverse transformation to either  $\beta$  or  $\gamma$  should be accomplished by the reconstructive mechanism. On the other hand, in shocked meteorites the common circumstance should be sudden transport into the  $\gamma$  stability field at low temperature and high stress (that is, by martensitic nucleation). Immediately thereafter, the stress and pressure will fall and the temperature will rise, in some cases leading to the  $\beta$ -phase before the reaction is quenched<sup>26-28</sup>. Downgoing lithospheric slabs present an environment in which both temperatures and stresses are intermediate between these other two environments. Under such conditions, the data are not yet sufficiently comprehensive to rule out either mechanism. We are continuing our experiments at intermediate temperatures and stresses to pursue this question.

It follows from these results that the level of stress could be a factor in experiments aimed at determination of the mechanism or kinetics of other transformations occurring in the deep Earth. In particular, if a martensitic mechanism is possible, then it is to be expected in shock experiments and also is likely in the laser-heated diamond cell. If such mechanisms are observed, their geophysical significance should be interpreted with caution. □

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## ESR dates for the hominid burial site of Es Skhul in Israel

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THE Middle East has been critical to our understanding of recent human evolution ever since the recovery of Neanderthal and early anatomically modern fossils from the caves of Tabun and Skhul (Mount Carmel) over 50 years ago<sup>1-3</sup>. It was generally believed, on archaeological and morphological grounds, that middle eastern Neanderthals (such as those from Tabun, Amud and Kebara) probably dated from more than 50,000 years ago, whereas the earliest anatomically modern specimens (from Skhul and Qafzeh) probably dated from about 40,000 years<sup>3</sup>. Recent thermoluminescence and electron spin resonance (ESR) determinations, however, have supported biostratigraphy in dating the Qafzeh deposits to an earlier part of the late Pleistocene, probably more than 90,000 years ago<sup>4-6</sup>. These dates have been questioned on unspecified technical grounds<sup>7,8</sup>, and it has also been argued that they create explanatory problems by separating the morphologically similar Qafzeh and Skhul samples by some 50,000 years, thus implying a long-term coexistence of early modern humans and Neanderthals in the area<sup>3,7,8</sup>. Here we report the first radiometric dating analysis for Skhul, using ESR on bovine teeth from the hominid burial levels. Early uptake and linear uptake ages average  $81 \pm 15$  and  $101 \pm 12$  kyr respectively. These analyses suggest that the Skhul and Qafzeh samples are of a similar age and therefore it is possible that the presence of early modern humans in the area was episodic, rather than long term during the early late Pleistocene.

The Israeli site of Es Skhul is located in the canyon of Nahal Mearot (Wadi el-Mughareh), near the site of Tabun which has yielded Neanderthal hominid remains. The site of Skhul originally consisted of a 2.5-m thick accumulation of densely cemented, reddish-brown breccia deposited on a triangular rock-cut platform about 11 m above the present level of the wadi floor. McCown<sup>9</sup>, who excavated the site, identified three successively older units. Layer A (<60 cm thick) contained a mixed assemblage of Middle and Upper Palaeolithic artefacts as well as some potsherds. Layer B (a breccia 2 m thick) contained the cranial and post-cranial remains of at least 10 hominids, the majority of which seem to have been intentionally buried, and over 9,800 lithic artefacts representing a Levallois-Mousterian (Middle Palaeolithic) industry. Layer C (a breccia <30-cm thick) contained a sparse industry similar to that in layer B, but no faunal material.

The hominids represent an archaic type of modern *Homo sapiens* and studies of their skeletal morphology demonstrate

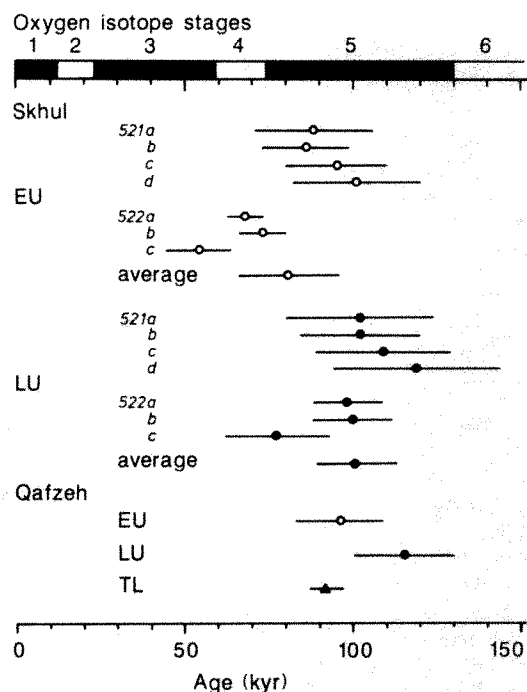


FIG. 1 Horizontal bars represent the ESR ages of two teeth from Skhul. Open circles, ESR ages according to early U-uptake; closed circles, ESR ages according to linear, continuous U-accumulation. The ESR and TL dates on Qafzeh are averages as given by Schwarcz *et al.*<sup>6</sup> and Valladas *et al.*<sup>4</sup>. The boundaries of the oxygen isotope stages were taken from Martinson *et al.*<sup>15</sup>.

clear affinities to those from Qafzeh cave, Israel<sup>3,10,11</sup>. To establish an absolute age for Skhul we made ESR measurements on two well-preserved bovid teeth from the British Museum (Natural History) collection. Although the exact positions from which the teeth were recovered is not recorded, it is known that they came from the relatively uniform layer B, which contained the hominids.

The procedures of ESR dating have been described by Grün

*et al.*<sup>12,13</sup>. The additive dose method was used to determine the acquired dose (AD). The U concentrations of enamel and dentine were determined by neutron activation analyses (Table 1). The external cosmic dose rate was calculated on the assumption that a maximum of 2 m of sediment covered the site. The external  $\beta$ -doses were estimated from analyses of the matrix adjacent to the teeth. Unfortunately, the enclosing matrix had been almost entirely removed by the excavators, so that it was not possible to measure the environmental  $\gamma$ -dose rate at the original site. We collected 16 samples of breccia matrix from the 'Mousterian Layers' of the original 1932 excavation as stored in the British Museum. These samples, plus the sediment samples adjacent to the teeth, were analysed for U ( $1.86 \pm 0.62$  p.p.m.), Th ( $2.18 \pm 1.31$  p.p.m.), and K ( $0.451 \pm 0.225\%$ ). These values correspond to a total external dose rate of  $507 \pm 123 \mu\text{Gy a}^{-1}$ , when including a cosmic dose rate of  $120 \pm 20 \mu\text{Gy a}^{-1}$  and a water content of  $10 \pm 10\%$ .

The present-day U content of the enamel ( $0.3\text{--}3.2$  p.p.m.) and dentine ( $6\text{--}12$  p.p.m.) must have been acquired post-mortem: in calculating the ESR age it can be assumed that U was taken up either soon after burial (early uptake, EU), or gradually (linear uptake, LU; see Fig. 1)<sup>12,13</sup>. For a given site, LU ages are generally closer to independently determined ages<sup>14</sup> than the minimum possible age defined by EU. The LU and EU ages of the present samples from Skhul average  $101,000 \pm 12,000$  years ( $101 \pm 12$  kyr) and  $81 \pm 15$  kyr, respectively. The former of these lies between the ESR (LU) and thermoluminescence (TL) dates obtained for Qafzeh ( $115 \pm 15$  kyr<sup>6</sup> and  $92 \pm 5$  kyr<sup>4</sup>, respectively) and is indicative of an early late-Pleistocene age for both sites, corresponding to oxygen isotope stage 5 of the deep-sea record<sup>15</sup>.

Schwarcz *et al.*<sup>16</sup> obtained U-series dates on stalagmitic calcite layers adhering to the bedrock or apparently interlaminated with the breccia. The youngest of a series of calcite layers was dated to  $79 \pm 4$  kyr. Although this agrees with the EU age, calcite may have continued to be deposited in this site after the burial was emplaced.

Previous indirect estimates of the age of this site have been based on comparisons of the lithic industry, fauna and hominid remains with those from other nearby sites, especially Tabun. Higgs<sup>17</sup> surmised, on the basis of a faunal analysis, that layer

TABLE 1 ESR results and analytical data for teeth from Skhul

Sample	U(EN) (p.p.m.)	U(DE) (p.p.m.)	U(SED) (p.p.m.)	Th(SED) (p.p.m.)	K(SED) (%)	Thickness* ( $\mu\text{m}$ )	Removed* ( $\mu\text{m}$ )
521a	0.3	6.0	2.3	1.3	0.27	1200	50
521b	0.3	7.3	2.3	1.3	0.27	1200	50
521c	0.2	7.1	2.2	1.3	0.27	1700	50
521d	0.3	7.5	2.2	1.3	0.27	1500	50
522a	3.2	11.3	3.8	4.2	0.79	1200	20
522b	2.0	10.7	3.8	4.2	0.79	1200	20
522c	3.0	11.5	3.8	4.2	0.79	1200	20

Sample	AD† (Gy)	$\gamma$ -(SED)‡ ( $\mu\text{Gy a}^{-1}$ )	$\beta$ -(SED)§   ( $\mu\text{Gy a}^{-1}$ )	$\beta$ -(DE)   ( $\mu\text{Gy a}^{-1}$ )	Early U-uptake		Age (kyr)	$\beta$ -(DE)   ( $\mu\text{Gy a}^{-1}$ )	Linear U-uptake		Age (kyr)
					Int.   ( $\mu\text{Gy a}^{-1}$ )	Total ( $\mu\text{Gy a}^{-1}$ )			Int.   ( $\mu\text{Gy a}^{-1}$ )	Total ( $\mu\text{Gy a}^{-1}$ )	
521a	$71.8 \pm 9.7$	$507 \pm 123$	$96.4 \pm 11.8$	121.3	90.5	$815.2 \pm 124$	$88.1 \pm 17.9$	57.0	40.5	$700.9 \pm 124$	$102.0 \pm 22.7$
521b	$72.3 \pm 2.6$	$507 \pm 123$	$96.4 \pm 11.8$	146.9	89.8	$840.1 \pm 124$	$86.1 \pm 13.1$	68.9	40.0	$712.3 \pm 124$	$102.0 \pm 18.1$
521c	$71.9 \pm 1.9$	$507 \pm 123$	$71.7 \pm 8.7$	115.2	64.0	$757.9 \pm 123$	$94.9 \pm 15.6$	53.9	28.4	$661.0 \pm 123$	$109.0 \pm 20.5$
521d	$82.7 \pm 9.4$	$507 \pm 123$	$79.3 \pm 9.7$	135.2	97.5	$807.6 \pm 123$	$101.0 \pm 19.0$	63.4	43.5	$693.2 \pm 123$	$119.0 \pm 25.1$
522a	$123.0 \pm 4.7$	$507 \pm 123$	$213.7 \pm 26.1$	217.9	869.2	$1,807.6 \pm 126$	$68.0 \pm 5.4$	107.3	422.7	$1,250.7 \pm 126$	$98.3 \pm 10.6$
522b	$108.7 \pm 4.8$	$507 \pm 123$	$213.7 \pm 26.1$	209.6	559.0	$1,489.3 \pm 127$	$73.0 \pm 7.0$	102.0	265.9	$1,088.6 \pm 126$	$99.9 \pm 12.4$
522c	$91.5 \pm 15.9$	$507 \pm 123$	$213.7 \pm 26.9$	211.3	745.3	$1,677.3 \pm 126$	$54.6 \pm 10.3$	104.0	361.2	$1,185.9 \pm 126$	$77.2 \pm 15.7$

EN=enamel; DE=dentine; SED=sediment;  $\beta$ -( )= $\beta$ -dose rate;  $\gamma$ -( )= $\gamma$ -dose rate; int.=internal dose rate.

\* The values for thickness of enamel and the layers removed from both sides (to eliminate the volume irradiated by external  $\alpha$ -rays) were used to calculate the  $\beta$ -attenuation factors<sup>22</sup>.

† ADs and uncertainties were calculated with the computer program FITT<sup>23</sup>.

‡ The uncertainty of the external  $\gamma$ -dose rate consists of the variation of radioactive elements in the sediment samples analysed by NAA and a systematic error for variation of water content<sup>4</sup>.

§ The uncertainty of the external  $\beta$ -dose is caused by a systematic error of water content<sup>4</sup>.

|| The analytical error in the determination of U, Th, and K is negligible for the calculation of the respective dose rates.

B at Skhul might be 10 kyr younger than layer C at Tabun, for which a  $^{14}\text{C}$  date of 51 kyr has been obtained<sup>18</sup>. Jelinek<sup>18</sup> compared the Tabun and Skhul artefacts and concluded that those from Skhul were less than 50 kyr old. Such estimates, as well as morphological dating, have been used to place the Skhul hominids at about 40 kyr<sup>1</sup>.

By contrast, our results show that the ESR dates for teeth associated with the Skhul hominids are indistinguishable from, or only slightly younger than those previously obtained<sup>6</sup> for anatomically similar hominids from Qafzeh. As at Qafzeh, the hominids are associated with a Middle Palaeolithic industry similar to that used at a later time by morphologically distinct Neanderthals elsewhere in this region<sup>19,20</sup>. It is unclear whether this precludes important behavioural differences between the early modern humans and Neanderthals in respects other than lithic industry. The Skhul and Qafzeh dates indicate the existence of anatomically modern hominids in south-west Asia long before their appearance in Europe, and at approximately the same time as their estimated earliest appearance in Africa<sup>5,21</sup>. The extent of any coexistence between early modern humans and Neanderthals in south-west Asia remains unknown, and documentation will depend on further dating work, particularly for Neanderthal sites such as Tabun, Amud and Shanidar. Clarification of the chronological and phylogenetic relationships between the Skhul-Qafzeh hominids and the more fragmentary and archaic humans known from sites such as Gesher Benot Ya'acov, Zuttiyeh and Tabun E is also required<sup>3</sup>.

An early late-Pleistocene age for both the Qafzeh and Skhul samples suggests at least two possibilities for this period of human evolution in south-west Asia. First, the presence of the early moderns might reflect a brief episodic occupation in the area (perhaps from north Africa), preceded by archaic humans associated with the Acheulo-Yabrudian, and followed by archaic humans (Neanderthals), also associated with the Middle Palaeolithic. Alternatively, archaic and early modern humans may have alternated or overlapped in occupation of the area through the Middle Palaeolithic. It remains unclear, however, whether the early late-Pleistocene presence of modern humans in south-west Asia presaged an early spread to east Asia (and much later, into Europe), or whether the further radiation of modern people into both Eurasia and the Far East was later, and unconnected with the evidence from Skhul and Qafzeh<sup>5</sup>. □

## A Middle Palaeolithic human hyoid bone

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THE origin of human language, and in particular the question of whether or not Neanderthal man was capable of language/speech, is of major interest to anthropologists but remains an area of great controversy<sup>1,2</sup>. Despite palaeoneurological evidence to the contrary<sup>3,4</sup>, many researchers hold to the view that Neanderthals were incapable of language/speech, basing their arguments largely on studies of laryngeal/basicranial morphology<sup>1,5,6</sup>. Studies, however, have been hampered by the absence of unambiguous fossil evidence. We now report the discovery of a well-preserved human hyoid bone from Middle Palaeolithic layers of Kebara Cave, Mount Carmel, Israel, dating from about 60,000 years BP. The bone is almost identical in size and shape to the hyoid of present-day populations, suggesting that there has been little or no change in the visceral skeleton (including the hyoid, middle ear ossicles, and inferentially the larynx) during the past 60,000 years of human evolution. We conclude that the morphological basis for human speech capability appears to have been fully developed during the Middle Palaeolithic.

The Kebara hyoid is part of a nearly complete Middle Palaeolithic skeleton (Kebara 2) unearthed during the 1983 joint French-Israeli excavations<sup>7,8</sup>. Figure 1 gives an anterior view of the bone. It is nearly complete—the body and the two greater horns are preserved. The latter are not fused to the body of the bone and the lesser horns are missing. Both of these features are also common in collections of modern human hyoids, because synostosis between the various elements of the hyoid is not always achieved, and in fact the small horns may remain cartilaginous during an individual's lifetime.

The ventral surface of the body presents two deep superior fossae separated by a median crest for the attachment of the geniohyoid muscle, and two less marked latero-inferior fossae for the omohyoid muscle. In a posterior (dorsal) view, the facets for the greater horns are very evident and the surface of the hyoid body is concave and rugged.

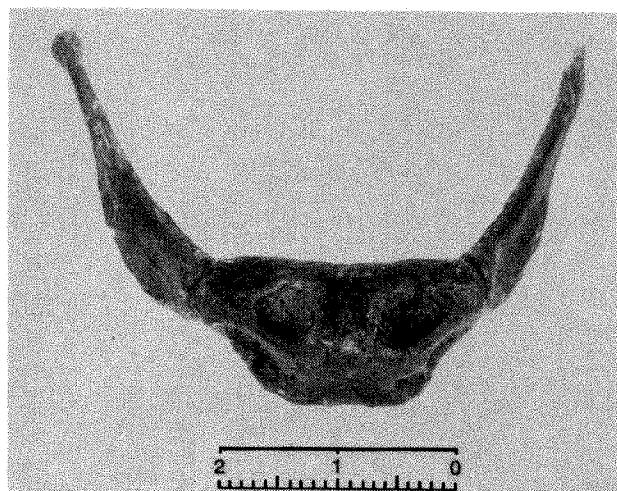


FIG. 1 The hyoid bone from Kebara 2 seen in anterior view.

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The right greater horn is complete and presents at its proximal end a large articular facet for the body of the hyoid, and at its distal end a small faint attachment surface for the thyrohyoid ligament. The left greater horn is missing its distal end. The principal measurements of the various elements of the hyoid bone are given in Table 1.

The metric traits of the Kebara bone are compared with the hyoid bones of a large sample of anatomically modern humans. The only consistent differences between the fossil bone and those of recent humans are in the maximum medial height of the body and depth of the posterior surface. Yet the former measurement is within the modern human range of variation; only the latter measurement exceeds the means and maximum range found in modern samples. The transverse diameter of the complete hyoid also appears to be large (Table 1), but the accurate position of the greater horns to the body is difficult to assess because of the missing articular cartilages.

Some other bones of the Kebara 2 skeleton present strongly marked metric and morphological differences with like bones of modern humans. For example, the mandible displays four dimensions (bicondylar breadth, bigonial breadth, bimental foramen breadth, and maximum body length) which are beyond

three standard deviations from the means of a sample of modern Bedouin mandibles. Indeed, the unusually large size of the Kebara 2 mandible implies a far greater robusticity of the muscles that relate this bone to the hyoid (for example, mylohyoid, geniohyoid, genioglossus, digastric) than in the Bedouin sample. The differences in the areas of attachment of muscles in Kebara 2 and modern humans are observed only in the mandible and are not reflected in the hyoid bone where insertions remain quite small, as in present-day populations. The hyoid of Kebara 2 thus presents a notable case of morphological stability through time, in sharp contrast to the mandible.

The relatively few morphological and metric changes that have occurred in the hyoid during the last 60,000 (and perhaps 90,000) years have been observed in other visceral bones. There is a marked similarity among the middle ear ossicles of Middle Palaeolithic Neanderthals, early anatomically modern humans, and present-day populations<sup>9-11</sup>. Together, the evidence from the hyoid and middle ear ossicles suggests that the osseous elements derived from the embryonic branchial arches show less rapid evolutionary changes than bones derived from dermal or enchondral tissues. A related inference would be that the associated larynx beneath the hyoid has scarcely changed in

TABLE 1 Measures (mm) of the Kebara hyoid bone compared with those of other groups

Group and period	Maximum transverse diameter*	Maximum medial height†	Anterior-posterior thickness‡	Depth of posterior surface§	Total sagittal diameter	Total transverse diameter¶
Kebara	24.6	13.4	5.8	3.8	35.5	45.0
Hayonim (Natufian)						
N	4	5	5	5	—	—
Mean + s.d.	22.00 0.73	11.04 1.17	5.78 0.69	2.28 0.29	—	—
Maximum	22.6	12.4	6.3	2.6	—	—
Minimum	21.0	10.0	4.6	1.8	—	—
La Boucle à Coronne (Neolithic)						
N	11	18	18	18	3	4
Mean + s.d.	20.74 1.56	10.37 0.92	4.85 0.72	2.03 0.68	33.33 2.90	38.78 1.31
Maximum	22.3	11.8	6.4	3.1	36.5	40.2
Minimum	18.3	8.5	3.7	0.8	30.8	37.2
La Cauna à Moux (Chalcolithic)						
N	5	12	10	11	—	—
Mean + s.d.	20.58 3.41	10.14 1.37	4.59 0.61	2.05 0.62	—	—
Maximum	23.6	11.7	5.3	3.0	—	—
Minimum	15.7	7.5	3.2	1.2	—	—
Ein Gedi (Roman period)						
N	15	15	15	15	—	—
Mean + s.d.	21.88 3.75	11.30 1.77	5.59 0.93	2.22 0.60	—	—
Maximum	27.2	14.2	7.8	3.4	—	—
Minimum	14.4	8.4	4.2	1.4	—	—
Bedouins (Recent period)						
N	13	17	17	17	8	8
Mean + s.d.	21.35 2.07	10.66 0.77	5.15 1.25	1.77 0.48	36.53 3.02	39.06 3.27
Maximum	25.4	12.0	9.0	2.5	39.8	43.5
Minimum	18.3	9.5	3.8	0.6	30.0	34.4
Total modern human sample						
N	48	67	65	66	11	12
Mean + s.d.	21.35 2.66	10.66 1.27	5.13 0.97	2.03 0.59	35.65 3.20	38.97 2.70

Samples from La Boucle à Coronne and La Cauna à Moux are from H. Duda excavations, housed in the Laboratoire d'Anthropologie, Université de Bordeaux 1, Talence, France. All other samples are from the Department of Anatomy and Anthropology, Tel Aviv University, Israel.

\* Maximum transverse diameter of body.

† Maximum medial height of body.

‡ Antero-posterior thickness of body in sagittal plane: one caliper branch tangent to the upper and to the lower posterior margins of the body, the other branch tangent to the most protruding antero-medial point.

§ Depth of the posterior surface of the body: measured from the upper and the lower posterior margins in the sagittal plane.

|| Total maximum length of the bone in the sagittal plane: in projection from distal end of the greater horns to most medio-anterior point of the body, with the greater horns being attached to the body in their anatomical position.

¶ Total maximum breadth of the bone in transverse plane: taken at the most external distal ends of the greater horns, with the greater horns attached to the body in their anatomical position.

position, form, relationships or size during the past 60,000 years of human evolution. If indeed this inference is warranted, the morphological basis for human speech capability appears to have been fully developed during the Middle Palaeolithic, contrary to the views of some researchers<sup>1,5,6</sup>.

The discovery of a fossil hyoid with strong similarities to hyoids of modern populations permits a new perspective on the Middle Palaeolithic human ability to communicate in an articulate language, with far-reaching implications regarding behaviour. The assumed speech limitations of the Neanderthals, that have hitherto been based primarily on studies of basicranial morphology, would seem to require revision. □

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## A cost of mating in female fruitflies

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**COSTS** of reproduction occur when an increase in reproductive rate reduces future reproduction by increasing mortality or reducing fertility. Such costs have been demonstrated in plants and animals in laboratory and field studies<sup>1–5</sup>. Their importance lies in their possible role in the evolution of life histories and of senescence<sup>6–15</sup>. An understanding of their mechanisms will also reveal the nature of the physiological constraints on longevity and fertility. Most accounts assume that these occur as a result of competition for nutrient allocation between growth, storage, somatic maintenance and reproduction<sup>16–21</sup>. An increase in reproductive rate would then result in a denial of nutrients to other processes, resulting in a drop in life expectancy or future fertility. Some support for this point of view comes from the finding that lifespan is lengthened in female *Drosophila melanogaster* that have inactive or absent ovaries<sup>22–23</sup> or that are experimentally induced to produce fewer eggs<sup>24</sup>. Increased exposure to males, however, also results in a drop in lifespan<sup>24–26</sup>. We show here that mating with males greatly reduces lifespan in female fruitflies whose rates of egg-production and egg-fertility do not differ, suggesting both that simple nutrient allocation to reproduction is not its only physiological cost, and that males can cause females to remate at a frequency that results in reduced female lifetime reproductive success.

Wild-type Dahomey virgin females were collected at eclosion from uncrowded cultures and allocated randomly to two experimental groups, each of 44 flies. The aim was to vary the opportunity for mating between the groups, but to equalize them for both production of fertile eggs and all aspects of pre-mating exposure to males. This was done by keeping each female in the 'high-mating' group permanently with two wild-type males,

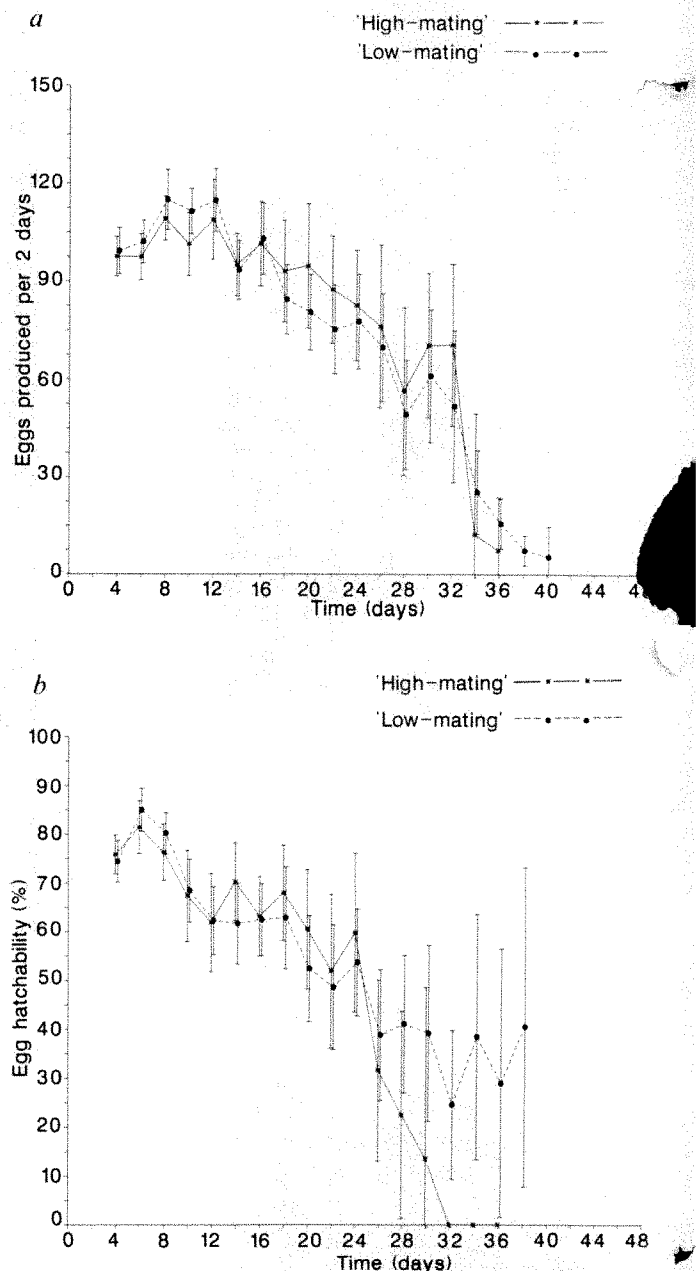


FIG. 1 a, Means (and 95% confidence limits) for the number of eggs produced in each 2-day interval. b, Means (and 95% confidence limits) egg-hatchability, measured for each female as the proportion of eggs laid producing adult progeny for each 2-day time interval.

initially 4-days old and renewed every 10 days. The females in the 'low-mating' group were kept with two wild-type males for 1 day out of 3, and on the remaining 2 days with two males that had had their external genitalia ablated by microcautery. These males courted normally (Table 1), and survived as well in the experiment as intact males, but they could not mate with the females; this was checked before the males were introduced to the experiment, by keeping them individually with two, initially 3-day-old, virgin females for 3 days, and checking for production of larvae. Exposure to microcauterized males resulted in levels of female activity that were not significantly different from those seen in females exposed to intact males (Table 2). All females were kept in 75×24 mm shell vials over 7 ml of freshly yeasted Lewis medium at 25°C on a 12:12 light-dark cycle, and were transferred to fresh vials every 2 days. All flies were handled under CO<sub>2</sub> anaesthesia. All eggs produced were

counted every 2 days, and the vials were retained for a count of the adults emerging, to give a measure of egg-hatchability. In a separate but identical experiment, the amount of mating by the two groups was measured on the one day in three when the 'low-mating' females were exposed to intact males.

The two groups of females showed no significant differences in the numbers of eggs produced (Fig. 1a), nor in the hatchability of eggs (Fig. 1b). The 'high-mating' group remated more frequently than the 'low-mating' group; on the day when both groups had intact males, the proportion of females remating did not differ significantly (Table 1). If these data are extrapolated to include the 2 out of 3 days when the 'low-mating' females had microcauterized males, females in the 'high-mating' group remated significantly more frequently. The 'high-mating' group had significantly lower lifespans than the 'low-mating' group (Fig. 2).

These results show that mating *per se* reduces female lifespan. The mechanism of the effect is at present unknown; it could be a consequence of mechanical injury, transfer of parasites, or an effect of sperm or accessory fluid components on the females. Transfer of parasites is an unlikely explanation, because the intact males used in the experiment were all virgins, and any non-venereal source of infection for males should also have been encountered by females. Use of mutant males may enable the mechanisms to be deduced. Our results suggest that diversion of nutrients into reproduction is unlikely to account for the reduction in lifespan, because the increase in mating was not accompanied by any increase in reproductive output. Females that lay more eggs remate more frequently<sup>27</sup>, so any increase in egg-production would be likely also to incur an additional mating cost.

The females in the 'high-mating' group suffered a drop in lifetime reproductive success as a result of their higher mating rate because their lifespan was reduced, but there was no increase in age-specific progeny production. They would therefore have had increased fitness if they had mated less frequently. Males always gain from a mating, and the finding implies that males can somehow persuade females to mate when it is not in their long term reproductive interests to do so. One possibility is that this is an artefact of the levels of exposure to males used in the experiment, so that the phenomenon would not occur in nature. The frequencies of remating that we have observed, however, are lower than those previously seen in natural or

TABLE 1 Numbers of females mating and courted

	Mating	Not mating
'High-mating'	15	264
'Low-mating'	25	983
	Courtship	No courtship
'High-mating'	97	173
'Low-mating'	108	166

Number of females mating in 33 h of direct observation in the 3 h after lights on, spread evenly over 11 days in the first 36 days of the experiment. For both groups the 'mating' total is the number of females mating on the sampling days. For the 'high-mating' group, the 'not mating' total is the number of females present on the sampling days that did not mate. For the 'low-mating' group, the 'not-mating' total is the number of females present on the sampling days that did not remate, plus the number of females present on the day before and the day after the sampling day.  $\chi^2$  1 d.f. = 6.1,  $P < 0.02$ . Lower part of table shows number of females courted in spot samples within 3 h of lights on and spread throughout the days of the experiment, on days when the 'low-mating' females were exposed to microcauterized males. Male courtship was defined as wing vibration or attempted copulation.  $\chi^2$  1 d.f. = 0.71,  $p > 0.4$ .

TABLE 2 Levels of female activity

	Courtied		Not courtied	
	Moving	Still	Moving	Still
'High mating'	24	73	28	145
'Low mating'	27	81	30	136

Spot samples of female movement (walking or running) while courtship was or was not occurring. Observations were spread evenly through the experiment, on days when the 'low-mating' females were exposed to microcauterized males. For courtship samples,  $\chi^2$  1 d.f. = 0.002 NS, for non-courtship samples  $\chi^2$  1 d.f. = 0.213 NS.

semi-natural populations<sup>28</sup>. This seems, therefore, to be a case where a conflict of interest between the sexes has been at least temporarily resolved in favour of males. This result does not support the idea that multiple mating may be of advantage to females<sup>29</sup>. □

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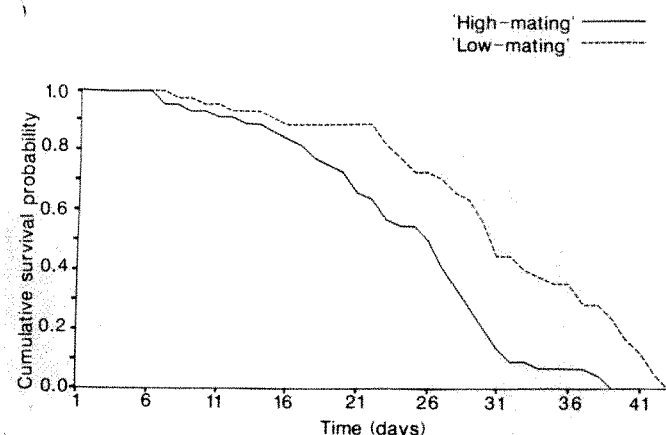


FIG. 2 Survival curves for 'high-mating' and 'low-mating' females. Cumulative survival probability is the proportion of females still alive at the end of each sampling interval.  $\chi^2$  1 d.f. = 15.4,  $P < 0.001$  Log Rank Test (this test compares the observed numbers of deaths in each group in each sampling interval with the numbers expected if the observed deaths occurred in proportion to the numbers of flies present in each group).



## 5-HT<sub>3</sub> receptors mediate inhibition of acetylcholine release in cortical tissue

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THE release of cerebral acetylcholine from terminals in the cerebral cortex has been shown to be regulated by 5-hydroxytryptamine (5-HT)<sup>1-6</sup> but it is not known which subtype of the 5-HT receptor is involved. 5-HT receptor agonists increase acetylcholine levels *in vivo*<sup>1,2</sup>, indicating a reduced turnover, and reduce release of acetylcholine from striatal slices *in vitro*<sup>3-5</sup>. Depleting 5-HT by inhibiting synthesis or by destroying the neurons containing 5-HT potentiates acetylcholine release<sup>5</sup>, and increases acetylcholine turnover in the cerebral cortex and hippocampus<sup>6</sup>. Selective antagonists for the 5-HT<sub>3</sub> receptor subtypes which seem to have effects on mood and activity<sup>7-10</sup> may exert their effect through the regulation of acetylcholine release in the cortex and limbic system. Radioligand binding studies show a high density of 5-HT<sub>3</sub> receptors in the cholinergic-rich entorhinal cortex<sup>11,12</sup> and we provide evidence that a reduction in cortical cholinergic function can be effected *in vitro* by 5-HT<sub>3</sub> receptors.

Release of [<sup>3</sup>H]acetylcholine from rat entorhinal cortex preloaded with [<sup>3</sup>H]choline was stimulated by potassium. Previous studies have shown that the tritium released is [<sup>3</sup>H]acetylcholine, and that the release is calcium-dependent<sup>13</sup>. 5-HT (2 µM) or the selective 5-HT<sub>3</sub> receptor agonist, 2-methyl-5-HT (ref. 14; 2 µM) inhibited potassium-stimulated [<sup>3</sup>H]acetylcholine release by some 50% (*P* < 0.001), but only in the presence of the 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptor antagonist ritanserin<sup>15</sup> (Table 1). Alone, the 5-HT<sub>3</sub> receptor antagonists GR38032F (ref. 16) and

zacopride<sup>17</sup> (both at 1 nM) failed to alter basal or evoked [<sup>3</sup>H]acetylcholine release, although they antagonized the reduction in evoked release induced by 2-methyl-5-HT (2 µM) in the presence of ritanserin (1 µM) (Table 1). In the absence of ritanserin, 2-methyl-5-HT in the presence of GR38032F or zacopride caused 107 and 37% increases in potassium-stimulated [<sup>3</sup>H]acetylcholine release, respectively (Table 1). The ritanserin sensitivity of this excitatory effect suggests mediation

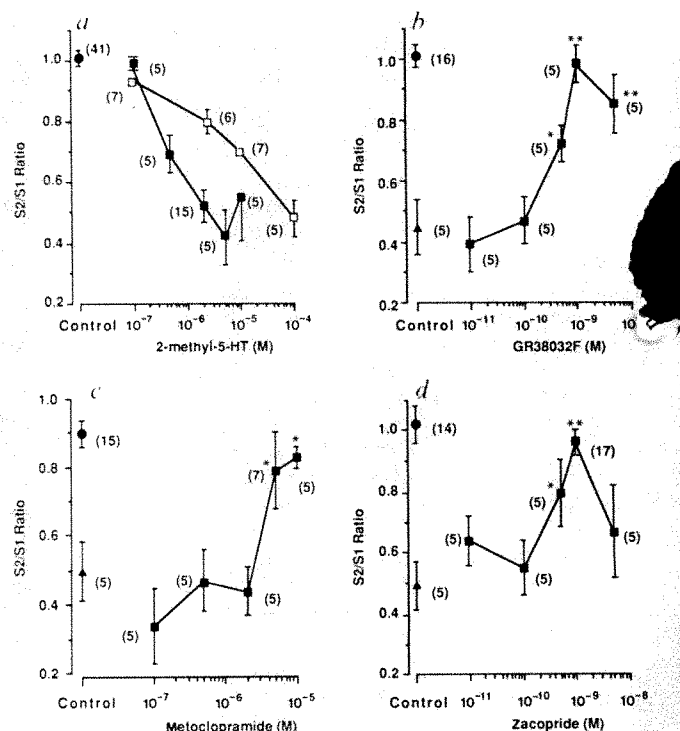


FIG. 1 Inhibition of potassium-stimulated [<sup>3</sup>H]acetylcholine release by 2-methyl-5-HT (0.1–10.0 µM) and antagonism by metoclopramide (0.1–10.0 µM), GR38032F (0.01–10.0 nM) or zacopride (0.01–10.0 nM). Ritanserin (1 µM) was used in all experiments. The entorhinal cortex from female, Hooded Lister rats (250–300g, Bradford bred) was cross-chopped at 90° (McIlwain tissue chopper) to produce slices 0.35 × 0.35 mm × thickness of cortical ribbon. Tissue was obtained from 20 rats for each experiment and each chamber contained tissue from a single rat. The cut tissue was washed three times in gassed (95% O<sub>2</sub>/5% CO<sub>2</sub>) Krebs solution (NaCl 118.0, KCl 4.75, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub> · 7H<sub>2</sub>O 1.2, CaCl<sub>2</sub> 2.5, NaHCO<sub>3</sub> 25.0, glucose 11.0 mM). The Krebs solution was then replaced by one containing 39.75 mM KCl (tonicity was maintained by reducing the NaCl concentration) and the tubes shaken at 37 °C for 20 min. After being washed the slices were incubated at 37 °C for 40 min in normal Krebs buffer containing [<sup>3</sup>H]choline, 0.1 µM (15 Ci mmol<sup>-1</sup>, Amersham). 200 µl washed settled slices were placed into each of the 20 superfusion baths and superfused with Krebs solution (0.5 ml min<sup>-1</sup>) containing 1.0 µM hemicholinium-3 at 37 °C. After a 30 min washout period, 4-min samples of perfusate were collected. At 12 min (S<sub>1</sub>) and 48 min (S<sub>2</sub>) the slices were depolarized by changing the superfusion fluid for 4 min to a Krebs solution containing 20 mM KCl. The superfusate samples were collected for a total of 80 min. Tritium content was assayed by liquid scintillation spectroscopy (Tri-Carb 1900CA, efficiency 47%). The evoked release of radioactivity was calculated as the difference between potassium-stimulated and basal release. On completion of the experiment, the tritium remaining in the slices was determined. The disintegration per min, per 4-min collection period, was converted to fractional release by dividing by the total amount of radioactivity present in the tissue at the end of each 4-min collection period<sup>13</sup>. Ordinates, mean S<sub>2</sub>/S<sub>1</sub> ratio ± s.e. calculated from *n* determinations. Abscissa, concentrations of agonist or antagonists. *a*, Concentration-response curves to 2-methyl-5-HT in the absence (■) and presence (□) of GR38032F (10 nM). *b*, *c* and *d*, Antagonism of 2-methyl-5-HT (▲, 2 µM) by GR38032F, metoclopramide and zacopride, respectively. Significant differences from 2-methyl-5-HT (2 µM) response are shown as \**P* < 0.05, \*\**P* < 0.01; ●, control response.

TABLE 1 Influence of 5-HT agonists and antagonists on [<sup>3</sup>H]acetylcholine release

Treatment	Evoked [ <sup>3</sup> H]acetylcholine release	
	Control	Treatment
5-HT	0.90 ± 0.03 (5)	0.77 ± 0.06 (4)
5-HT + Ritanserin	1.05 ± 0.03 (5)	0.51 ± 0.06 (4)*
2-Methyl-5-HT	0.98 ± 0.06 (5)	1.11 ± 0.14 (5)
2-Methyl-5-HT + Ritanserin	0.96 ± 0.06 (10)	0.52 ± 0.05 (15)*
Ritanserin	1.06 ± 0.07 (8)	0.91 ± 0.07 (5)
GR38032F	0.98 ± 0.06 (5)	1.04 ± 0.11 (3)
2-Methyl-5-HT + GR38032F	0.95 ± 0.03 (6)	1.97 ± 0.21 (6)*
2-Methyl-5-HT + GR38032F + Ritanserin	0.99 ± 0.04 (14)	0.98 ± 0.06 (12)†
Zacopride	1.01 ± 0.10 (5)	0.99 ± 0.05 (3)
2-Methyl-5-HT + Zacopride	0.98 ± 0.06 (5)	1.34 ± 0.15 (6)*
2-Methyl-5-HT + Zacopride + Ritanserin	1.01 ± 0.04 (14)	0.98 ± 0.04 (12)†

Influence of 5-HT agonists and antagonists, alone and in combination, on potassium-stimulated (20 mM) release of [<sup>3</sup>H]acetylcholine from slices of rat entorhinal cortex (see Fig. 1). All test drugs were added 20 min before, during and after the S<sub>2</sub> stimulation, and the S<sub>2</sub>/S<sub>1</sub> ratio was subsequently calculated: 5-HT (2 µM), 2-methyl-5-HT (2 µM), ritanserin (1 µM), GR38032F (1 nM), zacopride (1 nM). Mean S<sub>2</sub>/S<sub>1</sub> ratios ± s.e. and the numbers of rats used (*n*) for each determination are shown. Significant decreases or increases of S<sub>2</sub>/S<sub>1</sub> ratios compared with corresponding controls were determined using Dunnett's *t* test (*P* < 0.01\*). † Indicates significant antagonism of these effects by GR38032F, zacopride or ritanserin (*P* < 0.01).

through 5-HT<sub>2</sub> or 5-HT<sub>1C</sub> sites; although the involvement of 5-HT<sub>2</sub> receptors may be questioned as other functional studies have shown 2-methyl-5-HT to have little effect at the concentrations used in the present studies<sup>14</sup>.

The inhibitory effect of 2-methyl-5-HT on potassium-stimulated [<sup>3</sup>H]acetylcholine release was concentration-dependent in the 0.5–10.0  $\mu$ M range, which is typical of its potency for 5-HT<sub>3</sub> receptor-mediated responses<sup>14,16,18,19</sup>. Metoclopramide (5–10  $\mu$ M), GR38032F (0.5–5.0 nM) and zacopride (0.5–1.0 nM) showed a concentration-dependent antagonism on the inhibitory effect of 2-methyl-5-HT (2  $\mu$ M) (Fig. 1). The pIC<sub>50</sub> value ( $\pm$ s.e.) for zacopride, calculated from the rising portion of the antagonist response curve, was  $9.38 \pm 0.08$  (sample size,  $n = 5$ ) which is similar to reported values (5-HT<sub>3</sub> binding pK<sub>i</sub> = 8.70 (ref. 12), functional pA<sub>2</sub> values 8.5–10.1 (ref. 17)). The pIC<sub>50</sub> value for metoclopramide ( $5.80 \pm 0.09$ ;  $n = 5$ ), however, was slightly lower than expected (5-HT<sub>3</sub> binding pK<sub>i</sub> 6.45–6.49 (refs 11 and 12), functional pA<sub>2</sub> values 6.6–7.2 (refs 19 and 20)), which may reflect an antagonism of dopamine receptors.

In further concentration-response studies, GR38032F (10 nM) caused a parallel rightward shift in the 2-methyl-5-HT inhibitory concentration-response curve without changing the maximum response (dose ratio 15.6, Fig. 1). The competitive affinity (pK<sub>B</sub>  $\pm$  s.e.) for the antagonist GR38032F was  $9.04 \pm 0.12$  ( $n = 5$ ); this value is similar to those reported previously in functional systems (pA<sub>2</sub> 8.13–9.40 (ref. 16)) and binding assays (pK<sub>i</sub> 8.32–8.54 (refs 11 and 12)).

The specificity for 5-HT<sub>3</sub> receptors of the 2-methyl-5-HT (2.0  $\mu$ M) inhibitory effect (in the presence of 1  $\mu$ M ritanserin) was confirmed by the failure of other antagonists to influence the evoked [<sup>3</sup>H]acetylcholine release or its inhibition by 2-methyl-5-HT (namely, methysergide, fluphenazine, prazosin, propranolol, sulpiride, idazoxan, naloxone, atropine, mepyramine, ranitidine and chlordiazepoxide, all at 1  $\mu$ M).

Two concepts emerge from these *in vitro* studies. First, activation of 5-HT<sub>3</sub> receptors can reduce acetylcholine release from the cerebral cortex, and, second, 5-HT<sub>3</sub> receptor antagonists may act by facilitating acetylcholine release. Such a facilitation could be achieved either by directly preventing the inhibitory effect of a 5-HT<sub>3</sub>-receptor agonist or by allowing a facilitatory agonist to act at putative 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptors (ritanserin-sensitive sites). The increases in cortical acetylcholine release we report were effected by 5-HT receptor antagonists at affinities consistent with those determined in other 5-HT<sub>3</sub> receptor models (refs 11, 12, 16, 17, 19 and 20). GR38032F and zacopride, highly selective 5-HT<sub>3</sub> receptor antagonists, are particularly potent, and these novel psychotherapeutic agents may provide a link in the search for relieving the consequences of impaired cortical cholinergic function. □

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## Cytoadherence of knobless *Plasmodium falciparum*-infected erythrocytes and its inhibition by a human monoclonal antibody

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RED blood cells infected with mature stages of the malaria parasite *Plasmodium falciparum* bind to the endothelial lining of capillaries and venules. This sequestration is important for the survival of the parasite but may have severe consequences for the host. For example, it is involved in the causation of cerebral malaria which carries 25% mortality<sup>1–5</sup>. Knob-like protrusions present on the surface of infected erythrocytes have been considered necessary but not sufficient for this cytoadherence<sup>6–8</sup>. Here we describe the adhesion to endothelial cells of infected erythrocytes which do not have knobs. A human monoclonal antibody (33G2) which was specific for an epitope containing regularly spaced dimers of

glutamic acid present in the repeated amino-acid sequences of some defined *P. falciparum* antigens<sup>9,10</sup> was found to inhibit cytoadherence and may therefore be an important reagent for elucidating the molecular basis of parasite sequestration.

A bulk culture of the Palo Alto (PA) strain of *P. falciparum* bound poorly to the melanoma cell line C32 (100–200 infected red blood cells (RBC) per 100 melanoma cells). However, the number of binding RBC was significantly increased (2,000–3,000 RBC per 100 melanoma cells) after four consecutive adsorptions to melanoma cells and subsequent culture of the adherent infected RBC *in vitro* (Fig. 1). The cytoadherent RBC (C<sup>+</sup>) also bound to blood monocytes (1,000–1,200 RBC per 100 monocytes) and to endothelial cells from the human umbilical cord (100–3,400 RBC per 100 endothelial cells). The C<sup>+</sup> phenotype was stable and was retained for at least eight months, although at a somewhat lower level (600–800 RBC per 100 melanoma cells).

Previous studies of cytoadherence by transmission electron microscopy (TEM) have shown that the points of close contact between infected RBC and endothelial cells were knobs<sup>1–5,7,8</sup>. TEM showed that the C<sup>+</sup> cells, as well as those of the original PA culture we investigated, lacked knobs (K<sup>−</sup>) (Fig. 2a), and that the adhesion sites between RBC and melanoma cells were close and short alignments of the plasma membranes (Fig. 2b). When probed in immunofluorescence with the mouse monoclonal antibody 89, which is specific for the knob-associated antigen HRP-1 (KAHRP)<sup>11</sup>, the staining of K<sup>−</sup> C<sup>+</sup> RBC was essentially negative.

Like the melanoma-binding of K<sup>+</sup> cells<sup>6,12</sup>, that of K<sup>−</sup> cells was trypsin-sensitive (binding ~45% of control at 100  $\mu$ g ml<sup>−1</sup>) and was inhibited by the monoclonal antibody (mAb) OKM5 directed against the antigen CD36 on platelets, endothelial cells, melanoma cells and monocytes<sup>13,14</sup>. These results indicate that the molecular events involved in cytoadherence of K<sup>+</sup> and K<sup>−</sup>

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cells are similar or identical. But further experiments are needed to settle this question and also to establish the possible involvement of thrombospondin<sup>2,15</sup> in the cytoadherence of K<sup>-</sup> cells.

Only one of five immunoglobulins (IgGs) from *P. falciparum* immune donors inhibited cytoadherence of K<sup>-</sup> cells to melanoma cells (50% inhibition titre, 2 mg ml<sup>-1</sup>), although all

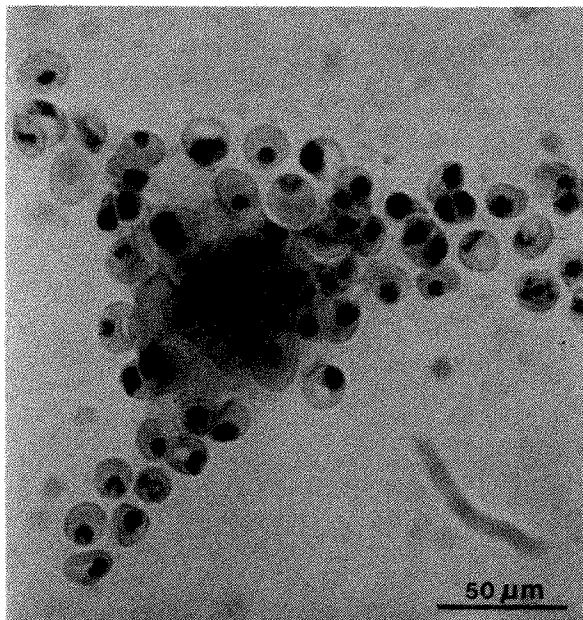


FIG. 1. Giemsa-stained *P. falciparum* RBC (Palo Alto) binding to melanoma cells (C32r).

**METHODS.** The cytoadherence assay was performed as described<sup>25</sup>. Briefly, melanoma cells grown on cover slips were fixed with 1% formaldehyde in phosphate-buffered saline (PBS) and stored at 4 °C until used. C<sup>+</sup> RBC (trophozoites/schizonts; 5–10% parasitaemia) were incubated with the melanoma cells at room temperature on a rotating platform for 1 h. Unbound RBC were flushed loose with PBS until only bound RBC remained. The coverslips were fixed with 1% glutaraldehyde in PBS, stained with Giemsa and examined in the light microscope.

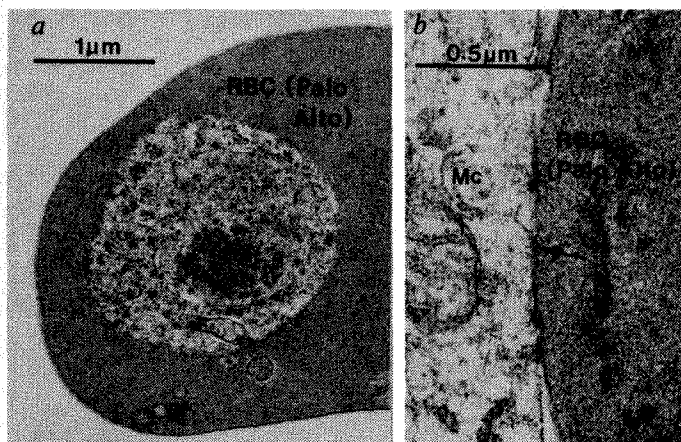


FIG. 2 *a*, Knobless *P. falciparum* RBC (Palo Alto) and *b*, binding (arrow) to a melanoma cell (Mc), as seen by transmission electron microscopy.

**METHODS.** Melanoma cells were grown in Petri dishes and fixed with 1% formaldehyde for 1 h. After immersion in PBS containing 0.2% BSA, they were scraped loose from the dishes. The cell suspensions were mixed with an equal volume of C<sup>+</sup> RBC (5–10% parasitaemia) and incubated under gentle rotation for 1 h at room temperature. The RBC/melanoma conjugates were collected by sedimentation and washed by addition of fresh PBS until most of the unbound RBC were removed. The sediments were fixed for 1 h at room temperature with 2.5% glutaraldehyde in 0.05M PBS (pH 7.4) containing 4% sucrose. After fixation, embedding and sectioning was as described<sup>26</sup>.

gave positive immunofluorescence with the surface of RBC containing late-stage parasites. But the human monoclonal antibody 33G2 (IgM,  $\kappa$ ) prepared from a malaria-immune donor<sup>16</sup>, strongly inhibited the binding of the K<sup>-</sup> cells in a dose-dependent manner (Fig. 3*b*). These results indicate either that the structures involved in cytoadherence of K<sup>-</sup> cells are weakly immunogenic, or that the corresponding antigens display a strain diversity similar to that seen in the cytoadherence of K<sup>+</sup> cells<sup>17,18</sup>.

The inhibitory human mAb 33G2 both stains the surface of suspended (unfixed) K<sup>-</sup>C<sup>+</sup>-PA cells in immunofluorescence and agglutinates these cells, indicating that there is a surface expression of the corresponding epitope. Staining, agglutination and cytoadherence inhibition were also obtained with K<sup>+</sup>-cultures of the Gambian *P. falciparum* strain FCR-3. Interestingly, RBC infected with PA parasites depleted of C<sup>+</sup> cells by repeated melanoma adsorptions were neither stained nor agglutinated by 33G2 and this was also true for K<sup>-</sup>C<sup>-</sup> cells infected with the Tanzanian strain F32. Taken together, these results suggest that the epitope seen by the 33G2 antibody is associated with cytoadherence of both K<sup>+</sup> and K<sup>-</sup> RBC.

Antibody 33G2 is specific for a repeated epitope consisting of regularly spaced dimers of glutamic acid in antigen 332, parasite-encoded polypeptide present in the membrane of RBC containing mature asexual stages of *P. falciparum*<sup>9,10</sup>. So far no

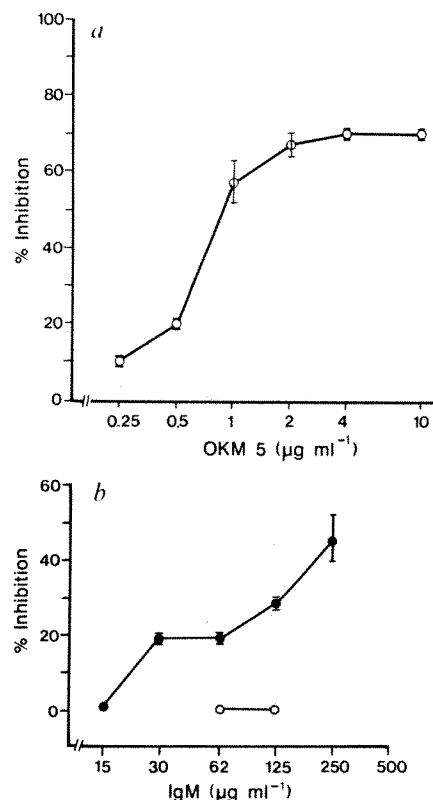


FIG. 3 Inhibition of cytoadherence by different antibodies. *a*, Inhibition by the monoclonal mouse antibody OKM5. *b*, Inhibition by the human monoclonal IgM antibody 33G2 (●). A human monoclonal IgM antibody specific for bacterial lipopolysaccharide (provided by Dr A. Rosén) was used as a control (○).

**METHODS.** *a*, Coverslips with formaldehyde-fixed melanoma cells (1% in PBS) were incubated with twofold dilutions of mAb OKM5 (0.25–10  $\mu\text{g ml}^{-1}$ ) for 30 min at room temperature. After three washes with PBS, C<sup>+</sup> RBC (Palo Alto) were added to the melanoma cells and the binding assay was performed as described (Fig. 1). The number of bound RBC per 100 melanoma cells was counted and expressed as the percentage of bound cells. *b*, Aliquots (0.5 ml) of concanavalin A-purified 33G2 (ref. 16) or of the control antibody were added to the pellets (15–250  $\mu\text{g ml}^{-1}$ ) and incubated for 30 min at 37 °C (total volume ~0.2 ml). One ml of PBS was added to each tube after incubation and the mixtures were then applied to the melanoma cells on coverslips. The binding assay and calculations were as in *a*. In each case, the mean and range % inhibition from two experiments are shown.



differences have been seen when lysates of  $C^+$  or  $C^-$  parasites separated by SDS-PAGE were probed with 33G2 in immunoblotting, and the possible relationship of antigen 332 to any of the polypeptides previously postulated to be associated with cytoadherence<sup>19-21</sup> is unknown. Antibody 33G2 cross-reacts with similar sequences of genetically unrelated *P. falciparum* antigens such as Pf155/RESA<sup>9,16</sup>, which cannot be implicated in cytoadherence<sup>19</sup>. But it also binds to Pf11.1 (refs 9, 22 and 23), a high-molecular weight polypeptide which may play a part in this process<sup>19</sup>. Thus it is not clear at present whether it is antigen 332, Pf11.1 or some other cross-reacting antigen which is responsible for cytoadherence-inhibition by antibody 33G2.

The present findings apparently conflict with earlier results, in so far as knobs at the surface of erythrocytes infected with asexual *P. falciparum* parasites have been considered necessary for cytoadherence. Sequestration of immature *P. falciparum* gametocytes has, however, been shown to occur independently of knob protrusions<sup>24</sup>. Similarly, sequestration of RBC containing asexual stages in other malarias (*P. knowlesi*, *P. berghei*, *P. chabaudi*) may also occur in the absence of knobs, whereas in other malarial species (*P. malariae*, *P. brasilianum*), infected RBC express knobs but do not cytoadhere<sup>19</sup>. Thus the presence of knobs seems necessary for cytoadherence of some parasite populations but not others. In any event, as the human monoclonal antibody 33G2 efficiently inhibits cytoadherence it may become a useful adjunct in the treatment or prophylaxis of severe *P. falciparum* malaria. □

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## A novel MHC class II epitope expressed in thymic medulla but not cortex

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THE repertoire of receptors expressed by peripheral T cells is the result of two selective events that occur during intrathymic development. Positive selection expands cells able to recognize foreign peptides presented by self MHC molecules, and negative selection eliminates cells reactive to self MHC molecules and associated self peptides<sup>1-4</sup>. Chimaera studies suggest that, at least in the case of T cells recognizing MHC class II, interaction with thymic cortical epithelial cells is responsible for the former<sup>5</sup>, whereas thymic medullary cells, of bone marrow origin, mediate the latter<sup>3,6-8</sup>. This view of thymic development is supported by recent morphometric analyses<sup>9</sup>, showing that autoreactive cells are found in thymic cortex but not medulla. Although numerous studies have shown that MHC class II molecules are expressed in both sites, none provides any explanation for the differential selection of T cells that is observed. Here, we describe a novel MHC class II epitope which is found on cells in thymic medulla but not cortex. The antibody to this epitope reacts with about 10% of class II molecules on B cells and may be recognizing a self peptide-MHC complex. These results provide the first evidence for differential expression of class II epitopes in different tissues and are compatible with the hypothesis that different ligands<sup>2,13-16</sup>,

rather than different affinity thresholds for the same ligand<sup>17,18</sup>, are involved in positive and negative selection of the T-cell repertoire.

The monoclonal antibody used in this study, Y-Ae, was produced by immunizing class II incompatible mice with lipopolysaccharide activated spleen cells (Fig. 1). The antibody specifically reacts with splenic B cells from strains which express I-A<sup>b</sup>(A<sup>b</sup>E<sup>b</sup>) and I-E<sup>b</sup>(E<sup>b</sup>E<sup>b</sup>) surface complexes, such as donor strain B10.A(5R) (Fig. 1a, b). It does not react with I-A molecules alone, as cells which express surface I-A<sup>b</sup> molecules, including strain B10.A(18R) spleen cells (Fig. 1a) and L cells transfected with A<sup>b</sup> and E<sup>b</sup> genes (Fig. 1c), are Y-Ae-negative. The antibody also does not react with I-E molecules, as several cell types that express surface I-E<sup>b</sup> complexes, including L cells transfected with E<sup>d/k</sup> and E<sup>b</sup> genes (Fig. 1d) and (B6.C-H-2<sup>bm-12</sup> × B10.TFR5)F1 spleen cells (Fig. 1h), are type Y-Ae-negative. However, both I-A and I-E genes influence the Y-Ae determinant. Formal proof that the E<sup>a</sup> gene or surface E<sup>a</sup>E<sup>b</sup> complexes are involved comes from the observation that Y-Ae reacts with spleen cells from B6-E<sup>d</sup> transgenic strain 107.1 (Fig. 1f) but not progenitor B6 (Fig. 1e). Studies with A<sup>b</sup> mutant strain B6.C-H-2<sup>bm-12</sup> provide clear evidence that at least the A<sup>b</sup> gene also influences Y-Ae activity, as shown by reactivity of the antibody with (B6 × BALB/c)F1 (Fig. 1g) but not (B6.C-H-2<sup>bm-12</sup> × B10.TFR5)F1 (Fig. 1h).

Evidence that the Y-Ae antibody binds to a modified form of I-A complexes comes from several sources. First, the antibody precipitates only mature forms of A<sup>a</sup> and A<sup>b</sup> chains from biosynthetically labelled spleen cell lysates (Fig. 2). No E<sup>a</sup> chains, E<sup>b</sup> chains, or invariant (Ii) polypeptides were observed in these immunoprecipitates. This formally excludes the possibility that the antibody reacts with mixed isotype E<sup>a</sup>A<sup>b</sup> dimers. Previous study showed that E<sup>a</sup> chains cannot pair with A<sup>b</sup> chains<sup>19</sup>. Failure to detect Ii chains suggests that the antibody reacts only with surface forms of class II molecules<sup>20</sup>. Second, several monoclonal antibodies<sup>21,22</sup> that react with sites influenced by  $\alpha$ -helical residues on A<sub>2</sub>(Y-3P, Y-248) or A<sub>2</sub>(Y-238) chains inhibit binding of Y-Ae to the lymphocyte surface, whereas monoclonal antibodies reactive with other parts of I-A molecules (Y-219) or with I-E molecules (Y-17, 14-4-4) failed

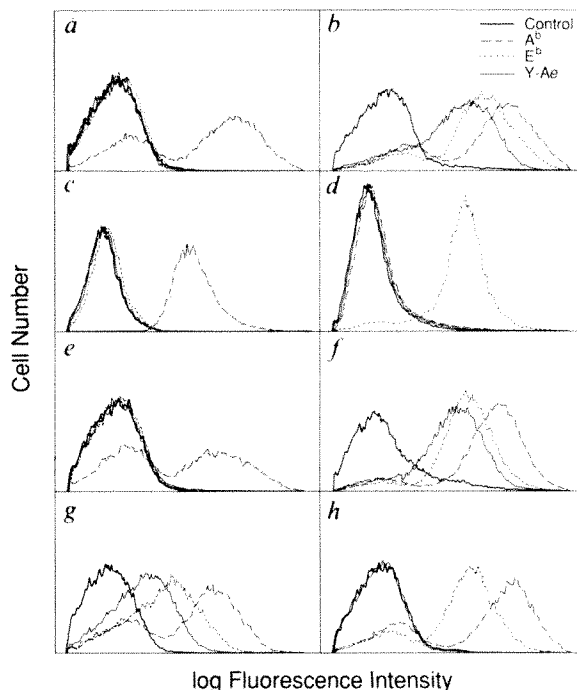
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to block (data not shown). Third, and most important, Y-Ae binds to about 10% of surface I-A molecules, as judged by competitive inhibition and quantitative binding analyses (Fig. 3). Taken together, these studies demonstrate that Y-Ae binds to a novel B-cell epitope on a subset of I-A<sup>b</sup> molecules in strains expressing surface I-E<sup>b</sup> molecules.

**FIG. 1** The Y-Ae antibody does not react with conventional I-A and I-E molecules, yet both I-A and I-E genes influence the Y-Ae determinant. Immunofluorescent analysis was carried out using the following biotin-conjugated monoclonal antibodies and cells. Control (—): anti-I-A<sup>b</sup>(A<sub>α</sub>A<sub>β</sub>), γ2b, 11.5.2 (ref. 24); A<sup>b</sup>(---): anti-I-A<sup>b</sup>(A<sub>α</sub>A<sub>β</sub>), γ2a, Y-3P (ref. 25); E<sup>b</sup>(· · · · ·): anti-I-E<sup>b</sup>(E<sub>α</sub>E<sub>β</sub>), γ2b, Y-17 (ref. 26); and Y-Ae (—): (B10.D2 × B10.MBR)/F1 anti-B10.A(5R) fused with Ag-8 and cloned by limiting dilution, γ2b. Panel a, B10.A(18R) spleen cells, which express A<sub>α</sub>A<sub>β</sub> surface molecules and E<sub>β</sub> cytoplasmic chains, but not E<sub>α</sub> chains. b, B10.A(5R) spleen cells which express A<sub>α</sub>A<sub>β</sub> and E<sub>α</sub>E<sub>β</sub> surface molecules. c and d, L cells transfected with A<sub>α</sub> and A<sub>β</sub> genes (c) or with E<sub>α</sub> and E<sub>β</sub> genes (d), gifts of Dr R. Germain<sup>27,28</sup>. e, B6 spleen cells, which possess a nonfunctional E<sub>α</sub> gene and express A<sub>α</sub>A<sub>β</sub> surface complexes and E<sub>β</sub> cytoplasmic chains. f, B6-E<sub>α</sub> transgenic strain 107-1 spleen cells, which possess a functional E<sub>α</sub> transgene and express A<sub>α</sub>A<sub>β</sub> and E<sub>α</sub>E<sub>β</sub> surface molecules in a normal manner. g, (B6 × BALB/c)F1 spleen cells which express A<sub>α</sub>A<sub>β</sub> and E<sub>α</sub>E<sub>β</sub> surface molecules. h, (B6.C-H-2<sup>bm-12</sup> × B10.TFR5)F1 spleen cells, which express mutant A<sub>α</sub>A<sub>β</sub><sup>bm-12</sup> and normal E<sub>α</sub>E<sub>β</sub> surface molecules. Similar results were obtained with (B6 × A.TFR5)F1(Y-Ae<sup>+</sup>) and (B6.C-H-2<sup>bm-12</sup> × A.TFR5)F1 (Y-Ae<sup>-</sup>) spleen cells (data not shown). Relevant difference between both sets of F1 hybrids is in the A<sub>β</sub> chain, where A<sub>β</sub><sup>bm-12</sup> differs from A<sub>β</sub><sup>bm-12</sup> at residues 67, 70 and 71 (ref. 29). In summary, the Y-Ae positive strains tested include: B10.A(5R), B10.A(3R), B10.BAR23, B10.BAR26, B10.BAR27, all 5R-like strains which express surface A<sub>α</sub>A<sub>β</sub> and E<sub>α</sub>E<sub>β</sub> complexes<sup>30</sup>, and B6-E<sub>α</sub> strain 107-1. The Y-Ae negative strains tested include: B6, B10, BALB.B, A.BY, B10.D2, BALB/c, B10.M, A.CA, B10.BR, BALB.K, C3H, B10.F(13R), B10.G, B10.RIII, B10.S, A.SW, B10.PL, B10.SM, B10.A, A, B10.A(2R), B10.A(4R), B10.A(18R), B10.MBR, B10.GD, A.TFR5, B10.TFR5, B10.HTT, B10.S(9R), B6.C-H-2<sup>bm-12</sup>, and B6-E<sub>α</sub> transgenic strain 36-5.

**METHODS.** Biotin-conjugated antibodies were added to spleen cell or L-cell suspensions for 30 min at 4 °C in phosphate-buffered saline containing 2% fetal calf serum, 0.1% sodium azide, and 1 mg ml<sup>-1</sup> normal mouse immunoglobulin to inhibit binding to Fc receptors (staining buffer). All antibodies were used at plateau staining concentrations. After washing with

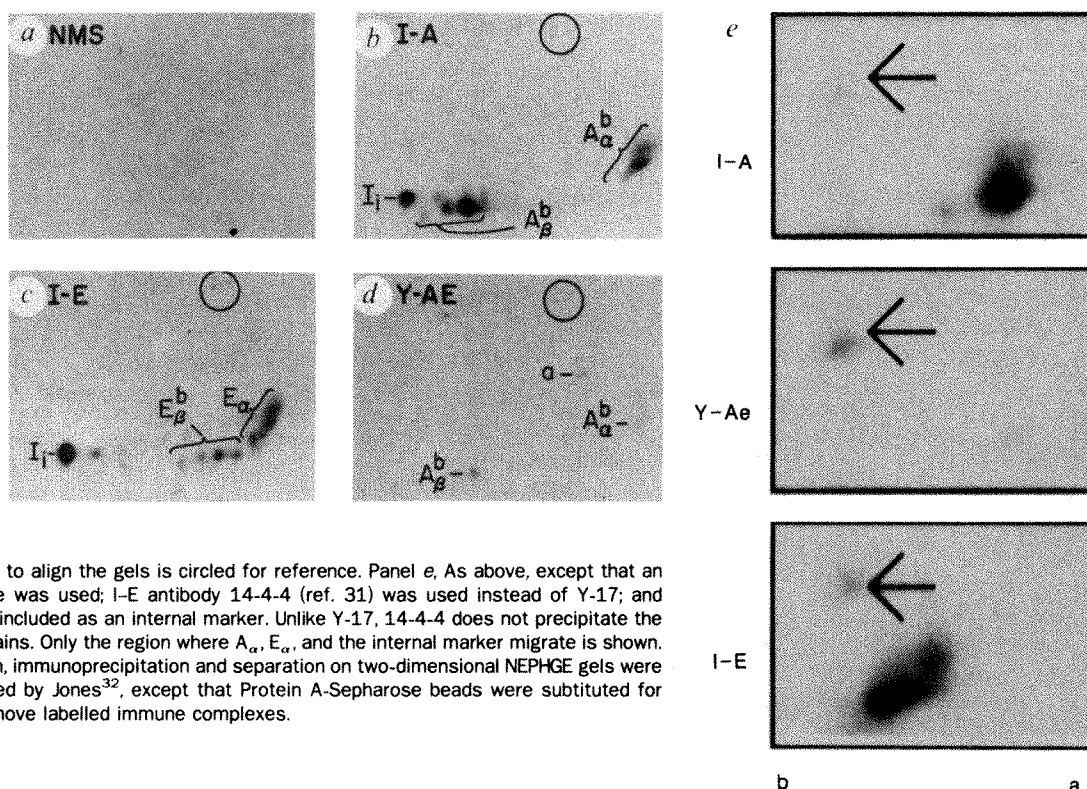
The epitope detected by Y-Ae on B cells is also strongly expressed on cells in the thymic medulla, but is not expressed on epithelial cells in the thymic cortex (Fig. 4). With both the B6-E<sub>α</sub> transgenic strain 107-1 and strain B10.A(5R) (data not shown), anti-I-A (Fig. 4a) and anti-I-E (Fig. 4b) antibodies give typical confluent staining of the medulla and reticular



staining buffer, cells were stained with FITC-Avidin for 20 min at 4 °C. Washed cells (20,000 per sample) were analysed on a FACS 440 (panels a, b, e-h) or a FACScan analyser (panels c, d). Cell number is displayed against a 3.7 log unit axis of fluorescence intensity.

**FIG. 2** The Y-Ae antibody reacts with mature forms of A<sub>α</sub> and A<sub>β</sub> chains. Panels a-d, two dimensional SDS-PAGE analysis of [<sup>35</sup>S]-methionine-labelled B10.A(5R) spleen cell lysates immunoprecipitated with normal mouse serum, anti-I-A (Y-3P), anti-I-E (Y-17) or Y-Ae. Note the identity but different intensity of the spots precipitated from the same lysate by Y-Ae and the major A<sub>α</sub> and A<sub>β</sub> spots precipitated by Y-3P; their distinction from all I-E spots precipitated by Y-17; and the absence of invariant chain (Ii) in the Y-Ae precipitate. A faint spot labelled (a) is actin. In the three immunoprecipitates, an

invariant triplet of spots used to align the gels is circled for reference. Panel e, As above, except that an independently prepared lysate was used; I-E antibody 14-4-4 (ref. 31) was used instead of Y-17; and <sup>14</sup>C-labelled OVA (arrow) was included as an internal marker. Unlike Y-17, 14-4-4 does not precipitate the most immature form of E<sub>α</sub> chains. Only the region where A<sub>α</sub>, E<sub>α</sub>, and the internal marker migrate is shown. **METHODS.** Labelling, extraction, immunoprecipitation and separation on two-dimensional NEPHGE gels were carried out exactly as specified by Jones<sup>32</sup>, except that Protein A-Sepharose beads were substituted for Staph A (Cowan strain) to remove labelled immune complexes.



staining of the cortex, whereas Y-Ae (Fig. 4c) reacts only with cells in the medulla. In contrast, the Y-Ae antibody does not react with any cells in the B6- $E^d$  transgenic strain 36-5 (Fig. 4f), which expresses I-A<sup>b</sup> molecules normally (Fig. 4d) and I-E<sup>b</sup> molecules on thymic epithelial cells only (Fig. 4e). This suggests that the Y-Ae epitope is expressed on bone marrow-derived cells in the thymic medulla.

Although it is clear that the Y-Ae antibody reacts with a subset of I-A molecules, and that an I-E gene influences the determinant recognized, the nature of the unique epitope detected is not yet known. We favour the interpretation that the antibody may be much like a T-cell receptor, detecting a self peptide: I-A complex. The most likely source of this peptide is the E<sub>α</sub> or E<sub>β</sub> chain. If the self-peptide: MHC model is correct, the antibody

will be extremely useful in analysing antigen processing at the molecular and ultrastructural levels. A second possibility is that the antibody reacts with a complex between I-A molecules and some intact protein whose expression is influenced by I-E molecules, such as the unknown products of *Mls* loci<sup>23</sup>. Other interpretations are also possible. To date, neither a processed peptide nor a third polypeptide chain has been detected by immunoprecipitation (Fig. 2).

The finding that a unique class II epitope is expressed on peripheral B cells and cells in the thymic medulla but not cortex is the first direct evidence that MHC molecules differ in conformation in a tissue-specific fashion. These results raise the possibility that the ligands involved in positive and negative selection of the T-cell repertoire may actually be different. If thymic

3 The Y-Ae antibody reacts with a subset of I-A<sup>b</sup> molecules on B10.A(5R) B cells. a, Unlabelled I-A<sup>b</sup> antibody Y-3P completely blocks binding of <sup>125</sup>I-labelled Y-Ae antibody to the B cell surface. b, Unlabelled Y-Ae partially inhibits binding of <sup>125</sup>I-labelled Y-3P. c, At saturation, the Y-Ae antibody binds at about 10% of the level obtained with Y-3P. d, The Y-Ae antibody binds with an avidity about 10-fold greater than that of Y-3P, as judged by Scatchard analysis. METHODS. T cells were depleted with anti-Thy-1 plus complement. Antibodies were <sup>125</sup>I-labelled, and binding assays carried out and analysed as previously described<sup>33</sup>, except that instead of washing extensively, cells were separated from unbound antibody by a single passage over oil-stop<sup>34</sup>.

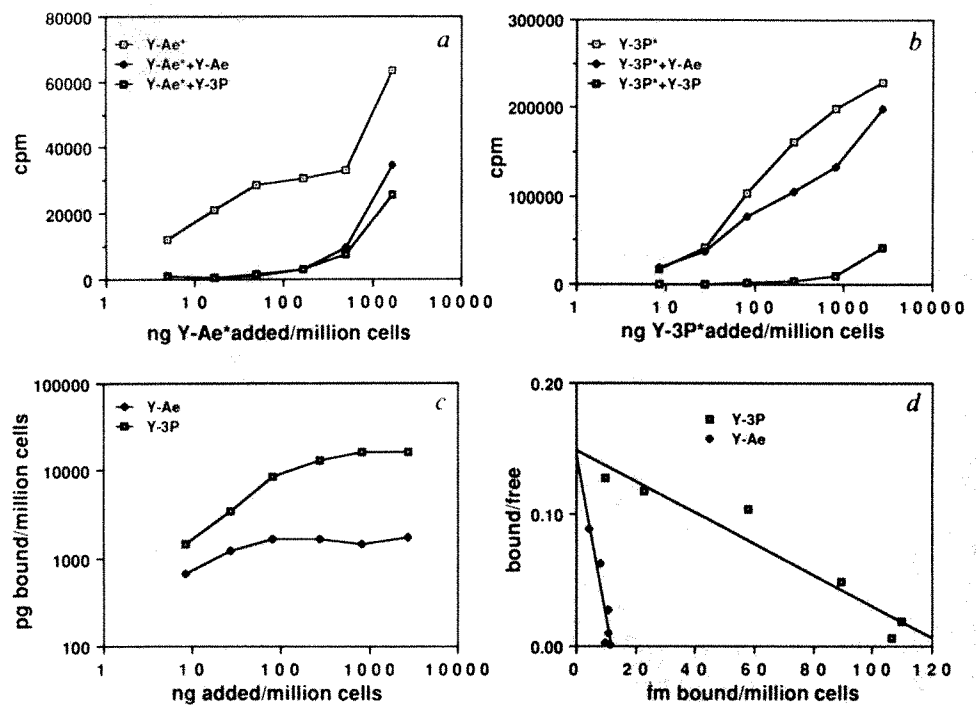


FIG. 4 The Y-Ae antibody reacts with cells in thymic medulla but not cortex. Immunoperoxidase staining of frozen thymus sections shows the distribution of I-A (panels a, d), I-E (panels b, e), and the determinant detected by Y-Ae (panels c, f) in different B6- $E^d$  transgenic mice. Strain 107-1 (panels a, c) expresses I-E molecules normally, whereas in strain 36-5 (panels d-f), I-E molecules are expressed only on thymic epithelium (compare panels b and e). Y-Ae stains the thymic medulla in strain 107-1, but does not stain thymic cortex in either strain, despite the presence of strong staining with both anti-I-A and anti-I-E antibodies. This result is independent of the concentration of antibody used, and was also obtained with B10.A(5R) mice (data not shown). Neither anti-I-E nor Y-Ae antibodies stain above the background seen in panel f with thymus sections from progenitor B6 mice or B6- $E^d$  transgenic mice expressing I-E molecules only in the pancreas (data not shown).

METHODS. Cryostat sections (10  $\mu$ m) of thymus from strain 107-1 or strain

36-5 were fixed in acetone and hydrated in PBS. Primary biotinylated antibodies, diluted in PBS with 30 mg ml<sup>-1</sup> BSA, were layered over the sections for 30 min. Sections were washed in phosphate buffered saline, and the bound biotinylated antibodies were detected using avidin-HRP (ABC-Elite, Vector labs) with DAB as the chromogen. Magnification factor: ~60-100 $\times$ . The derivation and characterization of the transgenic mice is described in refs 10 and 11.



medullary cells are involved in negative selection by clonal deletion<sup>1-4</sup>, then one can envisage T-cell development as occurring sequentially, with cells being positively selected for recognition of one set of class II epitopes on thymic epithelium, followed by the deletion of cells cross-reactive with unique epitopes found in the thymic medulla and on peripheral cells. □

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## Incubation time for AIDS from French transfusion-associated cases

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ALTHOUGH incubation time is a key parameter of the epidemiology of AIDS, statistical estimates based on transfusion-associated AIDS cases have, up to now, used only the single dataset provided by the AIDS program of the Centers for Disease Control (CDC) in Atlanta. Using a new dataset provided by the Direction Générale de la Santé (DGS), of the French Ministry of Health<sup>1</sup>, we estimate the mean incubation time for AIDS (median in brackets) to be 5.3 years (5.3 years) with a 90% confidence interval ranging from 4.4 to 8.9 years (4.4 to 8.8 years), when a Weibull distribution is postulated for incubation time. The previously encountered problem of very large confidence intervals (range larger than 100 years)<sup>2</sup>, is no longer observed, indicating that an accurate estimate for mean incubation time will be obtainable in the near future.

In France, the country with the second highest incidence of

AIDS cases<sup>3</sup>, 259 transfusion-associated cases, diagnosed before January 1988 by WHO criteria<sup>4</sup>, had been reported to the DGS by 25 March 1988. (Reporting AIDS cases to the DGS is mandatory in France.) Each case was individually reviewed by an epidemiologist to verify the quality of the data reported. Ninety-two cases associated with multiple transfusion, single transfusion without knowledge of the time of transfusion or other major transmission risk factors (homosexual or bisexual men, haemophiliacs, intravenous drug users) were eliminated, leading to a dataset of 167 subjects<sup>1</sup>. Due to the age-dependence of incubation time<sup>5,6</sup>, the analysis was further restricted to all subjects 13 years of age or older. Thus, the final dataset used to estimate mean and median incubation times for AIDS, and to obtain confidence intervals for these two parameters, comprised 148 subjects.

To analyse the data, we used the classical maximum likelihood method for which two derivations of the likelihood function that are applicable to the present problem have been described. The approach described by Medley *et al.*<sup>5,6</sup> is conditional on only  $N$ , the total number of transfusion-associated cases observed and allows for an exponential growth in infected transfusions before 1985 and a constant number since then. The method used by Lui *et al.*<sup>7,8</sup> is conditional on  $N$  and on the times  $t_1, t_2, \dots, t_N$  of observed infections, and provides a truncated likelihood<sup>9</sup> and as the derivation of confidence intervals is easier with the approach described by Lui *et al.*, the latter method was selected. A Weibull or a  $\gamma$ -distribution for incubation time were postulated as in several previous works<sup>2,5-8</sup>. To derive confidence intervals for mean and median incubation times for AIDS, the

TABLE 1 Expected and observed numbers of transfusion-associated AIDS cases by year of transfusion and year of diagnosis

Year of diagnosis	Year of transfusion									Total
	1978	1979	1980	1981	1982	1983	1984	1985		
1982	0.1	0.0	0.1	0.0	0.0	(expected)	1.0			0.2
	1	0	0	0	0	(observed)				1
1983	0.2	0.0	0.3	0.3	0.1	0.0				0.9
	0	0	0	1	0	0				1
1984	0.2	0.0	0.5	0.8	1.4	0.4	0.0			3.3
	0	0	0	0	3	2	0			5
1985	0.2	0.0	0.7	1.5	3.8	3.5	1.5	0.0		11.2
	0	0	1	1	2	2	3	0		9
1986	0.1	0.0	0.8	2.1	7.1	9.9	14.4	2.5		36.9
	0	0	2	2	3	17	18	3		45
1987	0.0	0.0	0.6	2.2	9.6	18.2	40.1	24.5		95.2
	0	0	0	3	14	11	35	24		87

The expected numbers are calculated using the Weibull distribution for incubation time.

likelihood ratio statistics (LRS), accepted as the best method in cases where data are truncated<sup>10</sup>, was used. Using a Weibull distribution, our point estimate for the mean (median) incubation time is 5.3 years (5.3 years). The 90% confidence interval is 4.4–8.9 years (4.4–8.8 years). The corresponding results with a  $\gamma$ -distribution are 6.9 years for the mean (6.4 years for the median) with a 90% confidence interval ranging from 4.9–19.0 years (4.6–17.1 years). An adjustment of the Weibull distribution to the data is presented in Table 1. Not surprisingly, the  $\chi^2$  value of comparison of the expected and observed values is not significant ( $\chi^2 = 7.0$ , 3 d.d.f.). A similar fit was observed with a  $\gamma$ -distribution, for the log of the likelihood value (see legend to Fig. 1), even if the confidence intervals for this distribution seem to be larger.

To compare our results with the most recent results obtained from the US dataset (by the method of Medley *et al.*<sup>11</sup>), the US data were analysed using the method described above<sup>7,8</sup>. The 543 cases reported to CDC by 8 April 1988, on the same selection criteria as for the French dataset, were included in the analysis. Our estimate of the mean, as compared with the last published analysis from Medley *et al.*<sup>11</sup>, was 7.3 years (90% confidence interval 5.7–11.7 years) versus 7.6 years, with a Weibull distribution and 9.8 years (90% confidence interval 7.1–19.0 years) versus 24.1 years with a  $\gamma$ -distribution. A large discrepancy between the results obtained by the two different methods is observed only when a  $\gamma$ -distribution is postulated. This distribution, however, was rejected by Medley *et al.*<sup>11</sup>, due to the very large annual rate of infective transfusions after 1985 that is predicted with their model with the  $\gamma$ -assumption. Moreover, the same authors now estimate<sup>12</sup> the mean incubation time at 14.3 years ( $\gamma$ -distribution), based on the April 1988 CDC data analysis. Unfortunately the authors do not comment on the sharp decrease (around 10 years) in their estimate.

The sizes of our confidence intervals seem relatively small compared with those reported by Kalbfleisch *et al.* (4.6,  $\infty$ ; ref. 2). Our explanation is that the precision of the estimate of the mean (or median) incubation time is related to the duration of the follow-up rather than to the number of subjects. To investigate this hypothesis, the French and US cases diagnosed before 1 January 1987 (61 and 388 cases, respectively) were analysed

using the Weibull distribution. This led, respectively, to a 90% confidence interval for the mean of (4.4,  $\infty$ ) and (5.7,  $\infty$ ).

In conclusion, when assuming a Weibull distribution for incubation time, the mean or median incubation time estimated from the French dataset, range from 4.4–8.9 years (90% confidence intervals). The evolution of the range of confidence intervals for the mean incubation time over the past year, clearly shows that reasonably narrow confidence intervals will be obtainable in the near future  $\square$

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## Paramyxovirus SV5 and multiple sclerosis

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MULTIPLE sclerosis is commonly associated with a local humoral immune response within the central nervous system. A hallmark of this intrathecal response is the presence of electrophoretically demonstrable oligoclonal bands of IgG in the cerebrospinal fluid (CSF) of up to 95% of patients<sup>1,2</sup>. Observations indicating that a major part of the CSF IgG in some patients may represent antibodies to SV5, a simian virus closely related to human parainfluenza type 2 virus, were recently reported by Goswami *et al.*<sup>3</sup>. We have studied thirty patients with multiple sclerosis, but although we find intrathecal synthesis of IgG antibodies reacting with SV5 in seven of these, the antibodies were not associated with oligoclonal CSF IgG bands and could in each case be explained as potentially cross-reacting antibodies to other paramyxoviruses known to be human pathogens. We have therefore been unable to confirm that SV5 may be a major intrathecal immunogen in multiple sclerosis.

Sera and CSF from thirty multiple sclerosis (MS) patients were analysed for IgG antibodies to SV5 and seven other viral antigens using an imprint immuno-fixation (IIF)<sup>4</sup> technique. In agreement with other reports<sup>5–7</sup>, intrathecal antibody responses to one or more viruses were observed in all thirty patients, seven of whom had intrathecal synthesis of antibodies reacting with SV5 (Fig. 1, Table 1). No evidence of intrathecal SV5 antibody synthesis was observed in six patients with defined neuroinfectious diseases (SSPE, HSV encephalitis, VSV encephalitis, tuberculous meningitis, Lyme neuroborreliosis, neurosyphilis) or in ten patients with neuroinflammatory diseases of undetermined aetiology.

Oligoclonal IgG bands were demonstrated by agarose gel electrophoresis and by IIF in the CSF from twenty-nine of the MS patients, including six of the seven with intrathecal SV5 antibody synthesis (Table 2). But the patterns of these bands did not correlate with those of intrathecally synthesized anti-

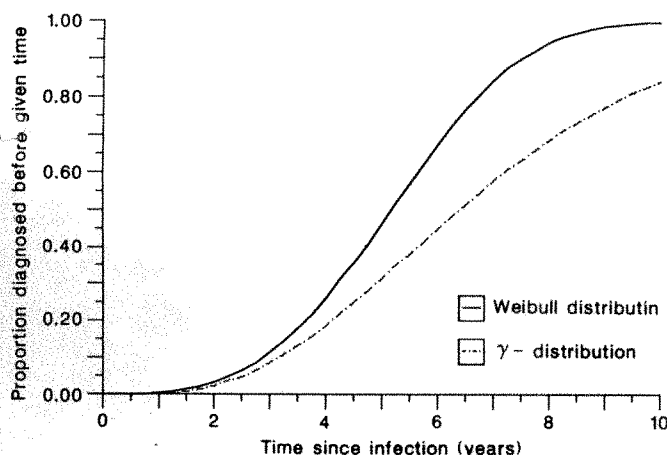


FIG. 1 The estimated incubation time described by Weibull distribution or  $\gamma$ -distribution shown as a proportion of transfusion-associated AIDS cases diagnosed before a given point in time. The 148 patients, 13 years of age or older, diagnosed before 1 January 1988 and reported to DGS by 25 March 1988 were included in the analysis. The density function according to the Weibull distribution is  $pq^p x^{p-1} e^{-(qx)^p}$ , where  $p$  and  $q$  are parameters, and  $x$  is the time between transfusion and diagnosis, the fitted parameters are  $p = 3.23$  and  $q = 0.17$  and the log likelihood is  $-509.203$ . The  $\gamma$ -distribution has the density function  $q^p x^{p-1} e^{-qx} / \Gamma(p)$ , the fitted parameters are  $p = 4.47$  and  $q = 0.65$  and the log likelihood is  $-508.596$ . The first parts of the curves were adjusted on the DGS dataset for up to 6.4 years (observed data) and then extrapolated from the model of the density function.

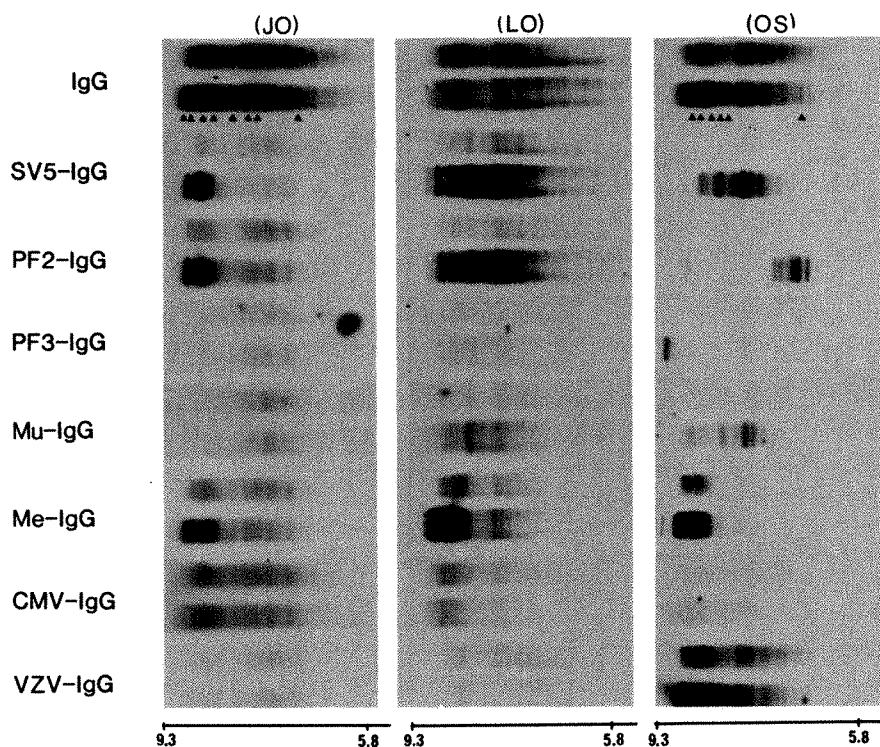


FIG.1 Imprint immunofixation (IIF) of total IgG and of IgG antibodies to SV5, parainfluenza (PF) viruses 2 and 3, mumps (Mu), measles (Me), varicella zoster (VZV) and cytomegalovirus (CMV) in electrofocussed (pH 3.5-9.5) pairs of serum and CSF from three patients with MS. Individual autoradiographs show serum above and CSF below. Note intrathecal synthesis of antibodies to SV5 and other viruses in each of the three patients (for criteria, see below) and of oligoclonal IgG bands (triangles) in two of the patients.

METHODS. SV5 (LN strain), PF2 (Greer strain), PF3 (C 243 strain) and mumps (Enders strain) cell pack antigens were prepared from infected Vero (African green monkey kidney) cell cultures. The infected cells were suspended in phosphate buffered saline at a concentration of ~10% (v/v), sonicated, and clarified by centrifugation (1,000 g for 5 min). Control antigen was prepared from non-infected Vero cells in the same way. Measles, HSV, VZV and CMV antigens were prepared as described previously<sup>7</sup>. Sera and concentrated CSF were adjusted to an IgG concentration of 2 g/dl. The procedure used for IIF has been described elsewhere<sup>4</sup>. The autoradiographs were evaluated visually. Fractions or 'bands' of IgG or of viral IgG antibodies in the CSF which had no, or clearly less intensely stained counterparts, in matching serum were concluded to derive from intrathecal synthesis.

bodies to SV5 or any of the other viruses (Figs 1, 2) and did not provide evidence that the oligoclonal IgG bands reacted with SV5.

The seven patients with intrathecal SV5 antibody synthesis generally had a higher number of intrathecally synthesized virus antibody specificities than those without, and each had intrathecal synthesis of antibodies to at least two of three other (PF2, PF3, mumps) paramyxoviruses (Table 2). In each case, a close correspondence was seen between CSF patterns of SV5 antibodies and antibodies to PF2 (5 patients) or mumps (2 patients) (Figs 1 and 2), suggesting cross-reacting activities between these antibodies. This was confirmed by virus absorption<sup>7</sup> of two selected CSF specimens, the first showing corresponding patterns of SV5 and PF2 antibodies and the second

of SV5 and mumps antibodies. Three separate 50  $\mu$ l aliquots of concentrated CSF (IgG concentration 1 g l<sup>-1</sup>) from each of the two patients were absorbed with 250  $\mu$ l SV5, PF2, or mumps antigen preparations respectively, and analysed by IIF (data not shown). SV5 antibodies were removed after absorption with SV5 and PF2, but not with mumps antigen, in the first specimen, and after absorption with SV5 and mumps but not with PF2 in the second. The IIF patterns of other viral (measles, HSV) antibodies and of oligoclonal IgG bands in the absorbed specimens were essentially unchanged. The removal of oligoclonal IgG bands after absorption with SV5, reported by Goswami *et al.*<sup>3</sup> in eight out of twelve patients, was therefore not confirmed here.

Using monoclonal antibodies, Randall *et al.* recently reported

TABLE 1 Viral IgG antibodies in serum and CSF and intrathecal antibody synthesis demonstrated by imprint immunofixation in 30 patients with MS

	Measles	HSV	VZV	Antibodies to CMV	Mumps	PF2	PF3	SV5
Serum	29	25	30	22	25	22	26	19
CSF	29	25	30	22	25	22	26	19
Intrathecal synthesis	24	6	18	0	15	12	15	7

For methods see legend to Fig. 1.

TABLE 2 Oligoclonal IgG bands in the CSF and intrathecal virus antibody synthesis in 30 patients with MS, segregated according to presence or absence of intrathecal synthesis of antibodies to SV5

Intrathecal synthesis SV5 antibodies	CSF IgG bands	Intrathecal antibody synthesis								No. of different virus antibody specificities*				
		Measles	HSV	VZV	CMV	Mumps	PF2	PF3	Any virus	1	2	3	4	5
Yes: N=7	6	6	2	5	0	6	6	6	7			1	1	5
No: N=23	23	18	4	13	0	9	6	9	23	6	4	6	5	2
Total: N=30	29	24	6	18	0	15	12	15	30	6	4	7	6	7

For methods see legend to Fig. 1.

\*SV5 antibodies excluded.



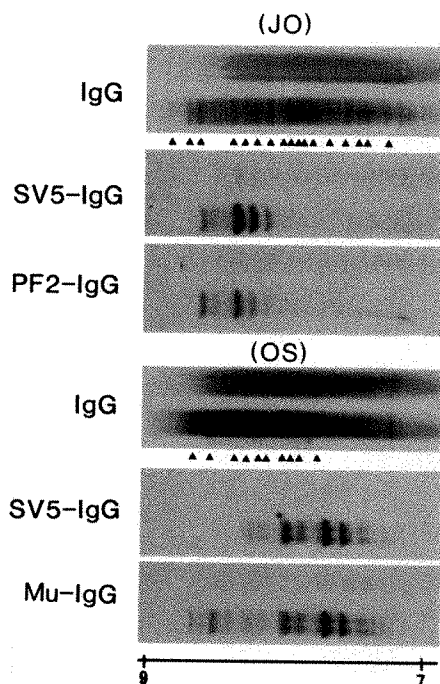


FIG. 2 Comparison of patterns of total IgG and viral antibodies in sera and CSF from two of the patients shown in Fig. 1. The specimens were electrofocused in a pH 6.5–9.5 gradient in order to provide better separation of IgG than in the pH 3.5–9.5 gradient shown in Fig. 1. Methods and symbols are otherwise as in Fig. 1. Note the difference in patterns between intrathecally synthesized SV5-IgG antibodies and bands of oligoclonal IgG (triangles) in both patients, and the correspondence between these antibodies and intrathecally synthesized antibodies to PF2 (case JO) or mumps (case OS).

the presence of cross-reacting epitopes on at least three virus structural proteins of SV5 and PF2, but not on the hemagglutinin-neuraminidase (HN) protein, which was unique to each virus<sup>8</sup>. Five of the monoclonal antibodies to SV5 HN used in this study (provided by Professor W. C. Russell) were analysed by IIF. Each reacted strongly with SV5 none reacted with PF2, showing that our method could readily detect antibodies to the SV5 HN protein. We conclude that methodological limitations do not explain the failure to detect antibodies reacting selectively with SV5 in the MS patients we studied.

In conclusion, our results suggest that there is no association between oligoclonal CSF IgG bands and SV5-specific antibodies in MS patients. The intrathecal synthesis of multiple viral antibodies constituting a minor fraction of the CSF IgG is generally thought to reflect a non-specific activation of B cells within the CNS, and is unlikely to be of aetiological importance. The oligoclonal IgG bands, however, represent a major fraction of the intrathecally synthesized immunoglobulin and may be recognizing major intrathecal immunogens involved in MS. Defining their specificity therefore remains a highly important challenge. □

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## Cloning of human telomeres by complementation in yeast

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TELOMERES confer stability on chromosomes by protecting them from degradation and recombination and by allowing complete replication of the end<sup>1</sup>. They are genetically important as they define the ends of the linkage map. Telomeres of lower eukaryotes contain short repeats consisting of a G-rich and a C-rich strand, the G-rich strand running 5'–3' towards the telomere and extending at the end<sup>2–11</sup>. Telomeres of human chromosomes share characteristics with those of lower eukaryotes<sup>12,13</sup>, including sequence similarity as detected by cross-hybridization<sup>14–16</sup>. Telomeric repeats from many organisms can provide telomere function in yeast<sup>2,17</sup>. Here we describe a modified yeast artificial chromosome (YAC) vector with only one telomere which we used to clone human telomeres by complementation in yeast. YACs containing human telomeres were identified by hybridization to an oligonucleotide of the trypanosome telomeric repeat. A subcloned human fragment from one such YAC is immediately subtelomeric on at least one human chromosome.

Human DNA digested with restriction enzymes which have four base-pair (bp) recognition sequences displays a range of fragments which hybridize to oligonucleotides containing the trypanosome telomeric repeat sequence TTAGGG. In the case of *Sau3A*, this range of fragments is centred at about 10 kilobases (kb) in DNA isolated from peripheral blood cells. These fragments are lost after brief digestion of intact DNA with

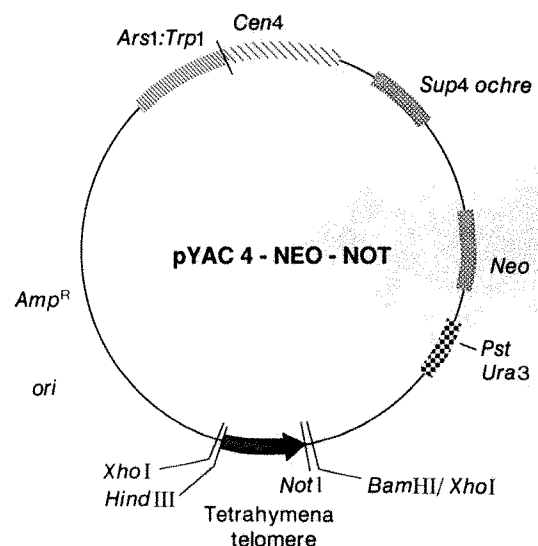


FIG. 1 Cloning of human sequences in pYAC4-Neo-Not. pYAC4-Neo<sup>16</sup> was cut to completion with *Bam*HI and partially with *Xho*I. The adaptor sequence 5'-TCGAGGGATCCGCGGCCG-3', base-paired with 15 of 19 bases, and having TCGA and GATC unpaired at the 5' ends, was ligated as described<sup>28</sup>. The adaptor destroys the original *Bam*HI site and recreates the *Xho*I site. The *his3* gene and one of the tetrahymena telomeric repeats (TTAGGG)<sub>n</sub> in pYAC4-Neo are deleted. A *Not*I restriction site is introduced adjacent to the remaining tetrahymena telomeric repeat and a *Bam*HI site between the *Not*I and *Xho*I sites. The structure shown was confirmed by restriction site mapping. Human placental DNA between 5 and 15 kb after *Sau3A* digestion was recovered from an agarose gel and ligated to pYAC4-Neo-Not which had been digested with *Not*I, dephosphorylated and digested with *Bam*HI. This DNA was used to transform AB1380 spheroplasts.

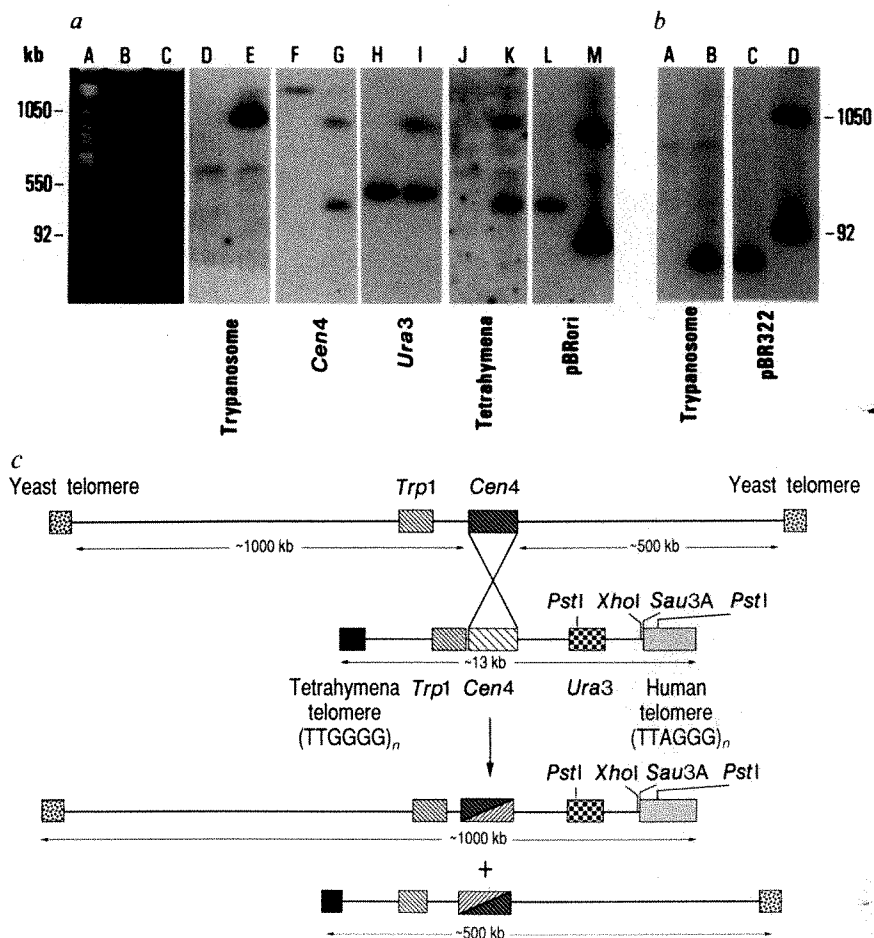
exonuclease *Bal31* (ref. 15). We have modified a YAC vector pYAC4 Neo (ref. 18) to give a vector (pYAC4-Neo-Not) which requires the addition of a second sequence with telomere function for its maintenance as a linear minichromosome in yeast. The presence of the *sup4* gene in the vector provides a colour test for the maintenance of this marker; AB1380 (ref. 19) yeast colonies are red in the absence and white in the presence of this gene. Details of the vector are given in the legend to Fig. 1. *Sau3A*-digested placental DNA from 5–15 kb was used as an enriched source of human telomeres for ligation to pYAC4-Neo-Not digested with *NotI* and *BamHI*.

AB1380 was transformed<sup>20</sup> with the ligation mix and selected for *Ura*<sup>+</sup> transformants. *Ura*<sup>+</sup> transformants (240) were screened for the presence of human telomeric sequences on the basis of colony hybridization to the oligonucleotide (TTAGGG)<sub>4</sub>. Two strongly hybridizing positive colonies were found (Hutel 1 and Hutel 22). Pulsed-field gel electrophoresis (PFG) comparison of AB1380 and Hutel 1 chromosomes showed that in this recombinant the 1.5 megabase (Mb) yeast chromosome 4 was replaced by two new chromosomes of 1.0 and 0.5 Mb respectively (Fig. 2a). Hybridization analysis shows that these chromosomes are the product of recombination between the transforming molecule and yeast chromosome 4 (Fig. 2a, c). In the case of Hutel 22, PFG analysis showed the presence of a 12-kb linear artificial chromosome, as shown in Fig. 2b. When plated on non-selective media, a high proportion of colonies were red as we expected, because a linear chromosome of this size should be relatively unstable<sup>21</sup>. In contrast, Hutel 1 gave less than 5 per cent red colonies when plated on non-selective media (data not shown).

FIG. 2 Novel yeast chromosomes with human telomeres. a, Hutel 1 and AB1380 analysed by pulsed field gel electrophoresis. A–C, ethidium bromide stained gel; D and E, probed with (TTAGGG)<sub>4</sub>; F and G, probed with a yeast *cen4* DNA fragment; H and I, probed with yeast *ura3*; J and K, probed with (TTGGGG)<sub>4</sub>; L and M, probed with pBR *ori* probe (*PvuII*–*PstI*). A and M, yeast marker chromosomes; B, D, F, H and J, AB1380; C, E, G, I, K and L, Hutel 1. The larger of the novel chromosomes hybridized to (TTAGGG)<sub>4</sub> and to *ura3*. Both novel chromosomes hybridized to a *cen4* probe and the smaller chromosome hybridized with the *ori* region of pBR322 and the tetrahymena telomere oligonucleotide probe (TTGGGG)<sub>4</sub>. Restriction analysis of Hutel 1 DNA showed that endogenous fragments were retained unchanged in size for the *trp1* and *ura3* genes, but that vector and endogenous *cen4* fragments were changed in size (data not shown). Homologous recombination between the *cen4* region of the vector and the original chromosome 4 would give rise to these chromosomes as detailed (Fig. 2c). b, Hutel 22 and AB1380 analysed by pulsed field gel electrophoresis. A and B probed with (TTAGGG)<sub>4</sub>; C and D probed with pBR322. A, AB1380; B and C, Hutel 22; D, YP148 marker chromosomes. Yeast DNAs were prepared in agarose plugs as described<sup>29</sup>. They were run on a 1% agarose gel overnight using a waltzer apparatus. The gel was made and run in 0.25 × TBE (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA). Conditions were 7 V cm<sup>-1</sup> at 10 °C and 90° between 120° reorientations. The gel was blotted to Hybond-N and hybridized with the <sup>32</sup>P-labelled probes as listed above. Hybridization with random primed fragments was overnight at 68 °C in 7% SDS, 0.5 M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 0.5% dried milk powder. Filters were washed in 0.1 × SSC, 0.1% SDS at 65 °C. Hybridization with oligonucleotides was overnight at 50 °C in 0.1% SDS, 0.1% sodium pyrophosphate, 0.05% bovine serum albumin, 0.05% polyvinylpyrrolidone, 0.05% Ficoll, 5 × SSC. Filters were washed in 4 × SSC, 0.1% SDS at 65 °C. Size markers (kb) are indicated. c, Recombination between

We wished to know if the human sequences in Hutel 1 and Hutel 22 were present at the end of the new yeast chromosomes. First, the end of a DNA molecule presents as an apparent site for all restriction enzymes. Such a site was present at the distal end of the region of human DNA homologous to the (TTAGGG)<sub>4</sub> oligonucleotide probe for both Hutel 1 and Hutel 22 (data not shown). Two additional features should be exhibited by terminal restriction fragments with human homology. They should be decreased in size and eventually become undetectable when intact recombinant yeast DNA is digested with exonuclease *Bal31*. Second, these terminal fragments should be heterogeneous in size when compared with fragments of the genome which are bounded by two restriction sites<sup>22</sup>. Figure 3 shows that both of these criteria are satisfied for Hutel 1. We consider that the most likely explanation for this heterogeneity is the addition of yeast terminal repeats. We conclude that Hutel 1 and Hutel 22 contain human DNA fragments with homology to trypanosome telomeric repeats and that these sequences support telomere function in a yeast.

Although >95 per cent of sequences which hybridize (TTAGGG)<sub>4</sub> are *Bal31*-sensitive in intact human DNA<sup>22</sup>, hence presumably telomeric or closely subtelomeric, it was important to show that the human sequences that we cloned in yeast are telomeric in the human genome. We have cloned some of the human sequences from Hutel 1 into a plasmid (pHutel 1), as described in the legend to Fig. 4. This cloned DNA fragment has no homology to the (TTAGGG)<sub>n</sub> terminal repeats detectable by hybridization or by sequence analysis (data not shown). When hybridized to blots of human DNA treated with *Bal31* for different times and then digested with *Sau3A*, this



endogenous and artificial chromosomes. Restriction sites relevant to the chromosome construction and subcloning are indicated. Figure is not to scale.

probe detected *Bal*31-sensitive fragments which were the same size as those detected by a (TTAGGG)<sub>4</sub> oligonucleotide probe (Fig. 4a). In addition, a number of discrete fragments are detected which do not correspond in size to fragments detected by the (TTAGGG)<sub>4</sub> probe. When hybridized to digests of blood and sperm DNA from different individuals, pHut1 DNA detected heterogeneous smears which were the same size as those detected by the oligonucleotide (TTAGGG)<sub>4</sub> and which were larger in sperm than in blood DNAs (Fig. 4b). This size

difference between these tissues from a single individual is a characteristic of the human pseudoautosomal telomeres<sup>13</sup>. From the restriction map presented in Fig. 2, we would predict that this probe would not detect heterogeneous fragments in a *Pst*I digest of human DNA. As shown in Fig. 4b, only discrete bands are detected in *Pst*I digests by pHut1. We conclude that this sequence must be immediately subtelomeric in the human genome.

Telomeres of all organisms studied consist of short tandemly

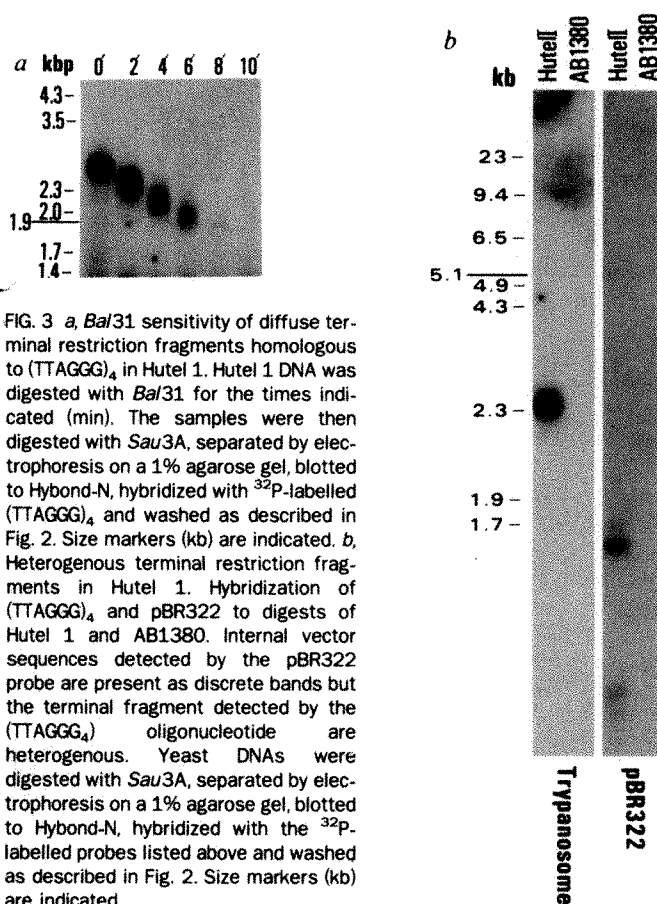


FIG. 3 a, *Bal*31 sensitivity of diffuse terminal restriction fragments homologous to (TTAGGG)<sub>4</sub> in Hutel 1. Hutel 1 DNA was digested with *Bal*31 for the times indicated (min). The samples were then digested with *Sau*3A, separated by electrophoresis on a 1% agarose gel, blotted to Hybond-N, hybridized with <sup>32</sup>P-labelled (TTAGGG)<sub>4</sub> and washed as described in Fig. 2. Size markers (kb) are indicated. b, Heterogenous terminal restriction fragments in Hutel 1. Hybridization of (TTAGGG)<sub>4</sub> and pBR322 to digests of Hutel 1 and AB1380. Internal vector sequences detected by the pBR322 probe are present as discrete bands but the terminal fragment detected by the (TTAGGG)<sub>4</sub> oligonucleotide are heterogenous. Yeast DNAs were digested with *Sau*3A, separated by electrophoresis on a 1% agarose gel, blotted to Hybond-N, hybridized with the <sup>32</sup>P-labelled probes listed above and washed as described in Fig. 2. Size markers (kb) are indicated.

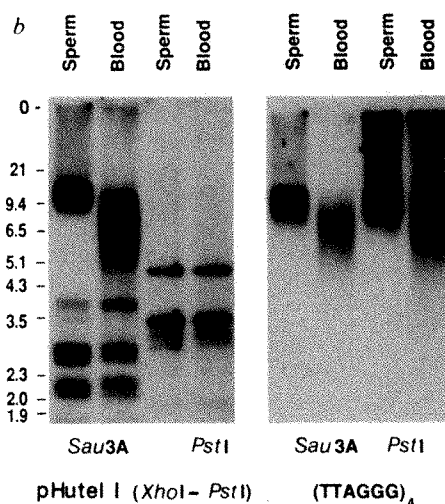
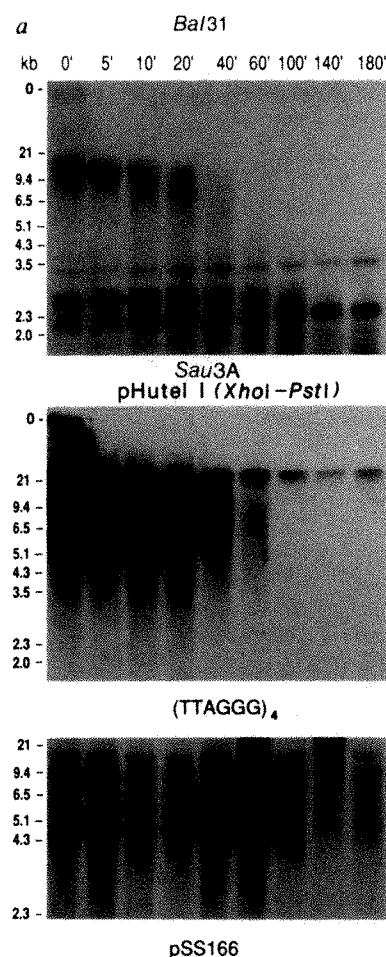


FIG. 4 Terminal location of pHut1 human sequences in the human genome. The human sequence in Hutel 1 was restriction mapped and a *Pst*I site proximal to the terminal repeats was found which, in the recombinant, lies on a *Pst*I fragment which also contains part of the yeast marker *ura3*. This fragment (pHut1) was recovered from a *Pst*I library of Hutel 1 in pTZ 19 using a *ura3* DNA fragment as a probe. a, Human placental DNA was digested with *Bal*31 for the times indicated (min). The samples were then split and digested with *Sau*3A or *Pst*I, separated by electrophoresis on 0.8% agarose gels and blotted to Hybond-N. The filter with the *Bal*31 series digested with *Sau*3A was first hybridized with <sup>32</sup>P-labelled *Xho*I-*Pst*I fragment containing human sequences from pHut1 and washed as described in Fig. 2. The filter was then stripped as recommended by the manufacturers, hybridized with <sup>32</sup>P-labelled (TTAGGG)<sub>4</sub> and washed as described in Fig. 2. The filter with the *Bal*31 series digested with *Pst*I was hybridized with <sup>32</sup>P-labelled pSS166 (*Hind*III fragment from cosmid B4 (ref. 30)). Hybridization was at 60 °C, but otherwise as described in Fig. 2. The filter was washed in 2 × SSC, 0.1% SDS at 65 °C. O, Gel origin; marker sizes are given in kb. b, Blood and sperm DNAs from the same individual were digested with *Sau*3A or *Pst*I, separated by electrophoresis on a 0.8% agarose gel, blotted to Hybond-N and hybridized with the <sup>32</sup>P-labelled probes as indicated. Hybridization and washing conditions were as described in Fig. 1. Size markers (kb) are indicated; O, gel origin.



repeated sequences. Variation in copy number of these repeats results in the heterogeneity observed for terminal restriction fragments<sup>23,24</sup>. In yeast the terminal repeats are the only elements required for telomere function<sup>22</sup>, and telomeric repeats from a number of organisms are capable of function in yeast by the addition of yeast sequences. Previous data<sup>12-15</sup> suggested that human telomeres showed many of the structural features of telomeres of lower eukaryotes. We have shown here that a telomeric fragment of human DNA can function as a telomere in yeast, suggesting that the structure of the ends of human chromosomes conforms to the general model of a number of short tandem repeats with a G-rich strand and a C-rich strand: the G-rich strand runs 5'-3' towards the end of the molecule and ends in a short single-strand extension. This degree of conservation and cross-kingdom function may reflect some similarities of replication mechanisms and protein interaction at the chromatin level. In other organisms there are proteins which interact specifically with the telomeres<sup>24,25</sup>. Alternatively, these sequences may themselves form functionally significant structures. G-G base pairing has been detected in tetrahymena telomeric sequences<sup>26</sup> and there may also be a requirement for the ability to form a four-stranded parallel base-paired structure in meiosis<sup>27</sup>.

Cloning of human telomeres in yeast provides a powerful method of obtaining markers at the ends of the physical and genetic maps of the human genome. Linkage of these markers to distal markers on the human linkage map will define the length of this map. □

## Molecular cloning of human telomeres in yeast

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**TELOMERES** are the DNA sequences found at the ends of linear chromosomes. They define the boundaries of the genetical and physical maps of such chromosomes and so are particularly important for the complete mapping of large genomes that is now being attempted. Telomeres have been intensively studied in the yeast *Saccharomyces cerevisiae* and in ciliated protozoa<sup>1</sup>: in these organisms the telomeric DNA consists of arrays of tandemly repeated short sequences in which one strand is guanosine-rich and oriented 5' to 3' towards the chromosome end. The conservation of these structural features is reflected in the observation that telomeric DNA from a variety of protozoa will function as telomeres on artificial linear mini-chromosomes in yeast<sup>1,2</sup>. Tandem arrays of the sequence TTAGGG have been identified at the telomeres of humans and other mammals<sup>3</sup> and also of trypanosomes<sup>4</sup>. This indicates that the structural features of telomeres are conserved between higher and lower eukaryotes and implies that human telomeric DNA could function in yeast. I have used this idea to develop a strategy to isolate a specific human telomere as a molecular clone in yeast and have devised a simple and effective way of cloning other human telomeres and their associated sequences.

Human telomeres contain the sequence (TTAGGG)<sub>n</sub>. This sequence is identical to the most prominent components of both the  $\alpha$ -satellite DNA of guinea pig<sup>5</sup> and the HS- $\alpha$ -satellite of DNA of kangaroo rat<sup>6</sup>. These satellite DNA sequences can be purified from the bulk of the genomic DNA by isopycnic centrifugation on a Cs<sub>2</sub>SO<sub>4</sub> gradient in the presence of silver ions, so human telomeric DNA could possibly be similarly purified. Accordingly, I restricted human placental DNA with *Bam*HI and fractionated it on such a gradient. Hybridization analysis (Fig. 1a) using the oligonucleotide probe (TTAGGG)<sub>4</sub> revealed

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that the telomeric DNA had been separated from the bulk of the genomic DNA. Fractions enriched in the sequence (TTAGGG)<sub>n</sub> were pooled and their enrichment with respect to the unfractionated DNA was calculated after gel electrophoresis, filter transfer and hybridization analysis (Fig. 1b). Densitometry indicated that these sequences had been enriched sevenfold with respect to the bulk of the genomic DNA.

The pseudoautosomal region of the human sex chromosomes includes a telomere. Sequences originating about 25 kilobase pairs (kb) from this telomere have been isolated as molecular clones in bacteria<sup>7</sup>. One of the sequences, 29cl, lies on the telomeric *Bam*HI fragment. I used this *Bam*HI fragment as a model to test whether a human telomere could function in the yeast *S. cerevisiae* by constructing a vector, pTV2 (Fig. 2a), for cloning telomeres in yeast. Digestion of this molecule with *Bam*HI and *Not*I generates two fragments: at one end of the larger is a sequence (denoted in Fig. 2a by 'TEL') that is derived from the ribosomal DNA of *Tetrahymena thermophila*<sup>8</sup> and which can function as a telomere in yeast, and at the other is a *Bam*HI site which could act as a cloning site. This molecule possesses only a single telomere and thus cannot autonomously function as a linear molecule in yeast cells. But if the telomere from the pseudoautosomal region can function as a telomere in yeast, then ligation of the *Bam*HI fragment (defined by 29cl) to the appropriately cut pTV2 will generate a molecule capable of autonomous linear existence in yeast. Accordingly, the telomere-enriched, *Bam*HI-cut DNA from the Ag<sup>+</sup>/Cs<sub>2</sub>SO<sub>4</sub> gradient was ligated to vector DNA cut with *Not*I and *Bam*HI and introduced into yeast. This generated ~2,600 transformants. (An undefined proportion of these transformants contain circular molecules derived from the large *Bam*HI/*Not*I fragment of pTV2 by illicit intramolecular ligation within the yeast, hence the complexity of the potential telomere library is as yet unknown.) The transformants were then divided into ten pools which were individually amplified and screened for the 29cl sequence by colony hybridization. Two pools each originating from the same transformation plate contained hybridizing colonies. These were isolated and examined for the presence of a linear mini-chromosome of the appropriate structure by digestion with restriction enzymes and pulsed-field gel electro-

FIG. 1 Enrichment of human telomeric DNA by isopycnic ultracentrifugation on  $\text{Ag}^+/\text{Cs}_2\text{SO}_4$  gradient. *a*, Spectrophotometric and hybridization analysis of fractions from the gradient. *b*, Hybridization analysis of DNA enriched for telomeric sequences.

**METHODS.** *a*, A solution of *Bam*HI-digested placental DNA (1.2 ml) at  $1.0 \text{ mg ml}^{-1}$  in  $0.1 \text{ M Na}_2\text{SO}_4$ ,  $0.1 \text{ M}$  borate buffer,  $\text{pH } 9.2$ , was slowly mixed with  $16.2 \text{ mM AgClO}_4$  to give a silver:phosphate molar ratio of  $0.2$ . This was added to a solution of  $\text{Cs}_2\text{SO}_4$  (density  $1.566 \text{ g ml}^{-1}$ ) in  $0.1 \text{ M Na}_2\text{SO}_4$  to give a final volume of  $24.7 \text{ ml}$ , a borate concentration of  $0.05 \text{ M}$  and a density of  $1.5 \text{ g ml}^{-1}$ . The mixture was centrifuged at  $50,000 \text{ r.p.m.}$  for  $50 \text{ h}$  in a Beckman VTi rotor. After centrifugation, the tube was pierced and  $0.24 \text{ ml}$  fractions collected from the bottom. Fractions were monitored spectrophotometrically and by dot-blot filter hybridization using a  $(\text{TTAGGG})_4$  probe. The hybridization and washing were carried out as described<sup>9</sup> at  $50^\circ\text{C}$ . The  $(\text{TTAGGG})_n$  sequence detected by this probe banded between  $1.55$  and  $1.53 \text{ g ml}^{-1}$ , which is consistent with the results obtained for guinea pig  $\alpha$ -satellite DNA<sup>10</sup>. *b*,  $5 \mu\text{g}$  human male *Bam*HI-digested placental DNA or  $0.7 \mu\text{g}$  telomere-enriched, *Bam*HI-digested DNA were electrophoresed on an  $18 \text{ cm}$ ,  $0.6\%$  agarose gel for  $16 \text{ h}$  at  $1.5 \text{ V cm}^{-1}$ . The gel was stained with ethidium bromide and photographed under ultraviolet light (left panel) and then filter-transferred and analysed with the  $(\text{TTAGGG})_4$  probe as described above (right panel).

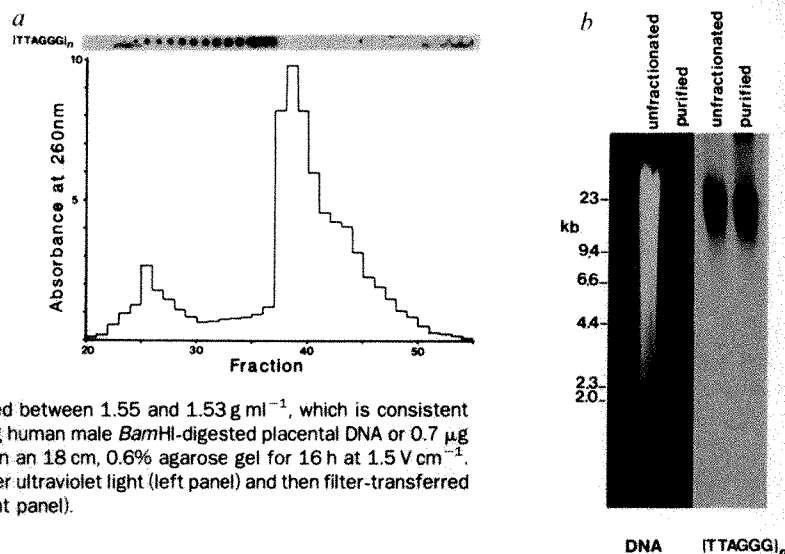
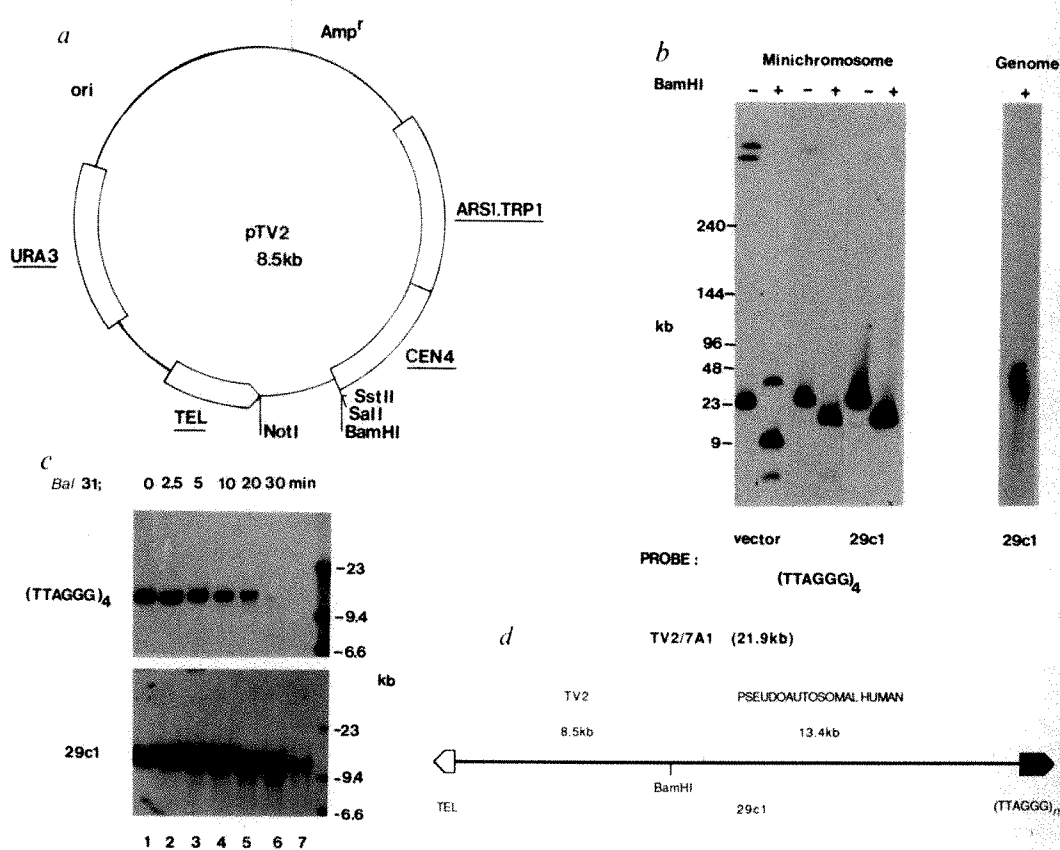


FIG. 2 Molecular cloning of the telomere from the human pseudoautosomal region and mapping of a minichromosome containing this telomere. *a*, Structure of pTV2. *b*, Restriction site analysis of the minichromosome TV2/7A1 containing the human pseudoautosomal telomere. *c*, Digestion with *Bal*31 and *Bam*HI reveal the relative orientation of 29c1 and  $(\text{TTAGGG})_n$  in TV2/7A1. *d*, Molecular map of TV2/7A1.

**METHODS.** *a*, pTV2 was constructed in *E. coli* from pYAC4<sup>11</sup>, a polylinker containing a *Not*I site and a polylinker containing *Bam*HI, *Sal*I and *Sst*II sites, using standard techniques. The vector and construction details are available on request. *b*, Telomere-enriched DNA ( $67 \mu\text{g}$ ) was prepared as described in the legend to Fig. 1 and ligated to  $25 \mu\text{g}$  dephosphorylated *Bam*HI-, *Not*I-cut pTV2 in  $300 \mu\text{l}$ . Unligated vector was removed from the ligation mix by preparative gel electrophoresis in low-gelling-temperature agarose (Seakem). Agarose containing the target DNA that was ligated to vector was melted and digested with Agarase (Calbiochem). The digest was extracted with phenol, then with chloroform and dialysed against  $0.01 \text{ M Tris.HCl}$ ,  $0.001 \text{ M EDTA}$ ,  $\text{pH } 7.4$ , and concentrated to  $300 \mu\text{l}$  by vacuum dialysis. The ligated DNA was then introduced into *S. cerevisiae* strain AB1380<sup>12</sup> by spheroplast transformation<sup>12</sup>. The transformants were selected on medium lacking uracil and collected in ten pools of  $\sim 200$  clones, then  $\sim 10,000$  cells from each pool were plated on to  $20 \text{ cm}$ -square Hybond-N membranes and screened by colony hybridization<sup>13,14</sup> using the 29c1 probe. Single colonies containing cognate DNA were then grown and used to prepare DNA in agarose plugs<sup>15</sup>. DNA from one clone that contained the minichromosome TV2/7A1 was analysed by pulsed-field gel electrophoresis<sup>16</sup> and filter-hybridization, either with (+) or without (-) prior *Bam*HI digestion. For comparative purposes, the *Bam*HI-digested male placental DNA ( $10 \mu\text{g}$ ) was run on the same gel. The probes used in the hybridization analysis were either the large *Xho*I-*Xho*I fragment of pYAC4 (vector), the oligonucleotide  $(\text{TTAGGG})_4$ , or 29c1. The 29c1 and the vector probes were used as described previously<sup>17</sup>. *c*, Agarose plugs containing  $\sim 1 \mu\text{g}$  yeast genomic DNA and the mini-chromosome TV2/7A1 in  $0.04 \text{ ml}$  were digested at  $30^\circ\text{C}$  for the time indicated with  $0.5$  units of *Bal*31 (BRL) in  $0.07 \text{ ml}$ . The digest



was stopped by adding  $1 \text{ ml}$   $0.02 \text{ M EGTA}$ ,  $0.01 \text{ M Tris.HCl}$ ,  $0.001 \text{ M EDTA}$ ,  $\text{pH } 7.4$ . After  $20 \text{ min}$ , the plug was rinsed in water, gelatin, salts and dithiothreitol as appropriate, and the DNA digested to completion with *Bam*HI. Before loading on to a  $0.8\%$  agarose gel the plugs were rinsed in  $10 \text{ mM Tris.HCl}$ ,  $1 \text{ mM EDTA}$ ,  $\text{pH } 7.4$ , and melted. The gels were ethidium bromide-stained and photographed under ultraviolet light to reveal the absence of any detectable endonuclease activity in the *Bal*31. They were then filter-transferred and analysed with the probes indicated, as described above. No DNA was loaded into track 7 of either gel, but in the gel shown in the lower panel, molten agarose leaked from track 6 into track 7 to give rise to hybridizing material in this track. Track 8 of each panel includes end-labelled *Hind*III fragments of phage  $\lambda$  DNA as size markers. *d*, When an exogenous telomere is introduced into yeast, it acts as the substrate for a terminal transferase that adds telomeric repeats which are characteristic of yeast to the pre-existing G-rich repeat<sup>18,19</sup>. The novel array characteristic of yeast typically extends for only a few hundred base pairs and is not illustrated on this map.

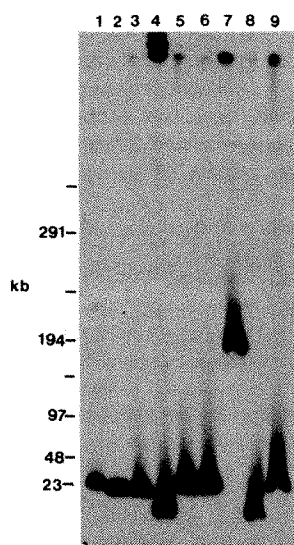


FIG. 3 Mini-chromosomes containing candidate human telomeres. The amplified population of transformants described in the legend to Fig. 2b was screened with the oligonucleotide (TTAGGG)<sub>4</sub>; clones that hybridized to this probe, but not to the 29cl sequence, were picked, cultured and used to prepare high-molecular weight DNA. These DNA samples were then analysed (tracks 2-9), together with that from the clone containing TV2/7A1 (track 1), by pulsed-field gel electrophoresis and filter-hybridization with the (TTAGGG)<sub>4</sub> probe. The two members of each pair of clones that migrate with identical mobility originate from distinct cloning events.

phoresis. They all contained a 22-kb molecule which is absent from the host strain and which hybridized to 29cl. The structure of the molecule, TV2/7A1, in cells derived from one colony was examined in more detail (Fig. 2b). TV2/7A1 hybridized to vector DNA, to 29cl and to the oligonucleotide (TTAGGG)<sub>4</sub> (Fig. 2b). *Bam*HI cleaved the molecule into two fragments whose sizes summed to that of the uncut minichromosome. One fragment of ~9 kb contained vector sequences (Fig. 2b), the other fragment of 13.4 kb contained the 29cl and (TTAGGG)<sub>4</sub> sequences (Fig. 2b). (In addition to the pTV2-derived fragment there are fragments in the *Bam*HI digest which hybridize to the vector; these result from hybridization to host genomic fragments.) If the (TTAGGG)<sub>n</sub> sequence on the pseudoautosomal fragment in the mini-chromosome has been recognized as a telomere by the yeast, then it should be susceptible to the action of the exonuclease *Bal*31. The 29cl sequence, on the other hand, should lie in the middle of the molecule and be relatively resistant to the action of this exonuclease. These predictions were tested by digesting DNA containing the mini-chromosome for progressively increasing lengths of time with *Bal*31, cutting with *Bam*HI, followed by Southern blotting and filter hybridization using either the (TTAGGG)<sub>4</sub> probe (Fig. 2c, upper panel) or the 29cl probe (Fig. 2c, lower panel). These results are summarized in the map in Fig. 2d. Sizing of the fragments produced by the action of *Bal*31 and *Bam*HI indicates that the (TTAGGG)<sub>n</sub> array extends for only 1.2 kb at the end of this mini-chromosome. But in human placental DNA, the sizes of the arrays with this sequence range from 9 kb to 20 kb (unpublished observations, and ref. 3). It thus seems likely that the (TTAGGG)<sub>n</sub> sequence is being abbreviated during the cloning procedure. This could be a consequence of the way in which the human telomere is healed in the yeast, although some exogenous tandemly repeated sequences are unstable in yeast artificial chromosome cloning vectors in a way that is reminiscent of their instability in *Escherichia coli* plasmid and phage vectors (C. Tyler-Smith and A. Villasante, personal communication). It is possible that such instability is contributing to the loss of these sequences from the chimaeric mini-chromosome. The observation (Fig. 2b) that the pseudoautosomal probe 29cl recognized a 30 kb fragment

in the *Bam*HI-cut placental DNA used for cloning is consistent with this explanation, although little is known about the structure of human proterminal DNA and more complex rearrangements could have occurred in the course of the cloning experiment. Further mapping experiments will resolve this point.

The observation that the pseudoautosomal telomere can function as a telomere in yeast and contains the sequence (TTAGGG)<sub>n</sub> indicates that other transformants in the pTV2 *Bam*HI-fragment library with this sequence could contain linear minichromosomes. I investigated this prediction by screening the amplified library with a (TTAGGG)<sub>4</sub> probe. Eight additional independent clones identified in this way were colony-purified and analysed by pulsed-field gel electrophoresis (Fig. 3). Each clone contains a minichromosome which includes (TTAGGG)<sub>n</sub>. The electrophoretic behaviour of these molecules indicated that they were probably linear and were between 10 and 200 kb long. Although there may be blocks of the (TTAGGG)<sub>n</sub> array elsewhere in the genome, it is reasonable to regard these molecules as candidates for novel unmapped human telomeres.

These results demonstrate a simple and novel strategy for isolating human telomeres and their flanking sequences. This will allow analysis of the structure and function of human proterminal DNA and will define the limits of the human genome map. In light of the evolutionary conservation of the distinctive structural and functional characteristics of telomeric DNA, the approaches described here could be applicable to a wide variety of organisms. □

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## Identification of the electron transfers in cytochrome oxidase that are coupled to proton-pumping

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**MITOCHONDRIAL cytochrome oxidase is a functionally complex, membrane-bound respiratory enzyme which catalyses both the reduction of O<sub>2</sub> to water and proton-pumping. During respiration, an exogenous donor, cytochrome c, donates four electrons to O<sub>2</sub> bound at the bimetallic haem a<sub>3</sub> Fe-Cu centre within the enzyme. These four electron transfers are mediated by the enzyme's haem a and Cu<sub>A</sub> redox centres and result in the translocation of four**



protons across the inner mitochondrial membrane.<sup>1</sup> The molecular mechanism of proton translocation has not yet been delineated, however, and in the absence of direct experimental evidence all four electron transfers have been assumed to couple equally to proton-pumping. Here, I report the effects of proton-motive force and membrane potential on two equilibria involving intermediates of the bimetallic centre at different levels of O<sub>2</sub> reduction.<sup>2-7</sup> The results show that only two of the electron transfers, to the 'peroxy' and 'oxyferryl' intermediates of the bimetallic centre, are linked to proton translocation, a finding which strongly constrains candidate mechanisms for proton-pumping.

The assumption that all four electron transfers from cytochrome *c* to O<sub>2</sub> bound to the bimetallic centre are equally coupled to proton-pumping (Fig. 1a) was tested by determining the linkage of two of the one-electron transfer reactions (Fig. 1b, box I) to proton and charge translocation. This was made possible by the observation that the catalytic cycle of the bimetallic centre can be driven in reverse, with discrete oxidation of intermediate O to F, and further to P, and concomitant reversed electron transfer to cytochrome *c*<sup>5-7</sup>. In isolated mitochondria, intermediates O, F and P can be brought in near equilibrium with cytochrome *c* (redox-poised with ferri-/ferrocyanide) by a proton-motive force across the membrane. Thus, the dependence of the P/F and F/O redox equilibria on proton-motive force and membrane potential ( $\Delta\psi$ ), at constant pH and redox potential of cytochrome *c*, provided information on the coupling of each electron transfer reaction to proton and charge translocation.

Figure 2 shows the dependence of the F/O and P/F redox equilibria on phosphorylation potential. Proton-motive force is generated by ATP hydrolysis which is catalysed by the mitochondrial H<sup>+</sup>-ATPase. The data indicate that electron transfer from cytochrome *c* to P, and to F, is coupled to the synthesis of 0.9–1.0 and 0.7–0.75 ATP molecules, respectively. This means that 1.6–1.75 ATP molecules are formed by oxidative phosphorylation coupled to the transfer of two electrons from cytochrome *c* to reduce P to O (through F). This 'local' ATP/2e<sup>-</sup> ratio is nearly twice that of overall cytochrome *c* oxidase activity<sup>8</sup>. It follows that the electron transfers from cytochrome *c* to P and to F constitute the main energy-conservation steps in the catalytic cycle of cytochrome oxidase. By contrast, the two remaining electron transfers, from cytochrome *c* to O and to H (Fig. 1b) make only a small contribution to ATP synthesis, which may be limited to the electrogenicity of electron transfer from cytochrome *c* to the bimetallic centre (Fig. 1a).

A quantitative evaluation of the linkage of the P/F and F/O reactions to proton and charge translocation requires a knowledge of the number of protons translocated per hydrolysed extramitochondrial ATP. At present an H<sup>+</sup>/ATP ratio of 4 is widely accepted<sup>9</sup>. From Fig. 2 it therefore follows that the P/F and F/O transitions are coupled to the overall translocation of 3.6–4.0 and 2.8–3.0 H<sup>+</sup>, respectively. It should be noted, however, that two events other than proton-pumping are electrogenic in the oxidase reaction (Fig. 1a). The first is electron transfer between cytochrome *c* and the binuclear centre; the

second is the uptake of the protons from the inside of the inner mitochondrial membrane that are consumed in reduction of O<sub>2</sub> to water<sup>10</sup>.

Table 1 summarizes the quantitative analysis of the results shown in Fig. 2, from which it can be concluded that both the P/F and the F/O transition are coupled to the pumping of close to 2 H<sup>+</sup>.

Figure 3 shows the dependence of the F/O equilibrium on electrical membrane potential at high pH, where the intermedi-

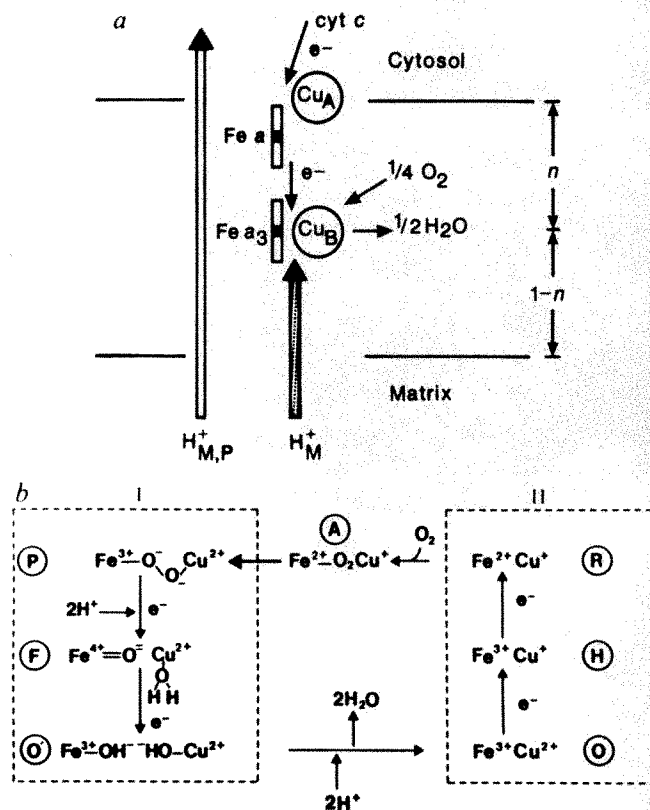


FIG. 1 a, Electrogenic reactions of cytochrome oxidase. Each electron transferred from cytochrome *c*, through haem *a* (Fe *a*) and Cu<sub>A</sub>, to the bimetallic haem *a*<sub>3</sub> (Fe *a*<sub>3</sub>)-Cu<sub>B</sub> centre, and reduction of O<sub>2</sub> to water, is accompanied by the uptake of 1 H<sup>+</sup> from the matrix phase for the formation of water (shaded arrow), and the pumping of 1 H<sup>+</sup> across the membrane (white arrow). The fraction of the total transmembrane dielectric traversed by electron transfer from cytochrome *c* to the bimetallic centre is denoted by *n*. Proton uptake to the centre traverses the fraction (1 - *n*). b, Proposed catalytic scheme of O<sub>2</sub> reduction by the bimetallic centre. Fe and Cu denote the iron of haem *a*<sub>3</sub> and Cu<sub>B</sub>, respectively. The main intermediates previously observed spectroscopically are shown. O and O' are not distinguishable by optical spectroscopy, but by the pH-dependence of the O → F transition. Uptake of H<sup>+</sup> corresponds to H<sub>M</sub><sup>+</sup> in a. Proton-pumping is not shown. Dashed boxes labelled I and II outline the parts of the cycle that are here proposed to be coupled and not coupled to proton-pumping, respectively. Further details are described in refs 5–7, 10.

TABLE 1 Calculation of the H<sup>+</sup>/e<sup>-</sup> ratio of proton-pumping

Reaction step	H <sup>+</sup> uptake in reaction H <sub>M</sub> <sup>+</sup>	Charge translocated in reaction q <sup>+</sup>	Observed total H <sup>+</sup> translocated H <sub>obs</sub> <sup>+</sup>	H <sup>+</sup> pumped H <sub>M,P</sub> <sup>+</sup>	Charge pumped q <sub>P</sub> <sup>+</sup>
F → O (pH 7.2)	1	1	2.8–3.0	1.8–2.0	1.8–2.0
P → F (pH 8.3)	2	1.5	3.6–4.0	1.6–2.0	2.1–2.5

H<sub>M</sub><sup>+</sup> is the number of H<sup>+</sup> taken up from the matrix side of the membrane at the indicated pH, excluding proton-pumping, as determined previously<sup>7</sup> (see Fig. 1a, b). q<sup>+</sup> is the number of electrical charge equivalents translocated, excluding proton-pumping, linked to the corresponding reaction, and assuming *n* = 0.5 (Fig. 1a). H<sub>obs</sub><sup>+</sup> denotes the observed total number of H<sup>+</sup> translocated (Fig. 2), assuming a H<sup>+</sup>/ATP ratio of 4. H<sub>M,P</sub><sup>+</sup> is the number of H<sup>+</sup> taken up from the matrix side due to proton-pumping alone (Fig. 1a), obtained as H<sub>obs</sub><sup>+</sup> - H<sub>M</sub><sup>+</sup>. q<sub>P</sub><sup>+</sup> is the number of electrical charge equivalents translocated due to proton-pumping alone, obtained as H<sub>obs</sub><sup>+</sup> - q<sup>+</sup>. Due to inequivalency of q<sup>+</sup> and H<sub>M</sub><sup>+</sup>, and the dominance of the  $\Delta\psi$  term over the  $\Delta\text{pH}$  term of the proton-motive force, H<sub>M,P</sub><sup>+</sup> underestimates, and q<sub>P</sub><sup>+</sup> overestimates the H<sup>+</sup> pump stoichiometry for the P → F transition.

ate O' dominates over O (ref. 7) (Fig. 1b) so that the only linked electrogenic event expected apart from proton-pumping is electron transfer to cytochrome *c*. The 27-mV dependence observed indicates that the oxidation of O' to F by cytochrome *c* is coupled to the translocation of  $\sim 2.2$  (60 mV/27 mV) charge equivalents. Subtraction of the fraction due to electron transfer (see legend to Table 1 and Fig. 1a) yields 1.7 charges translocated by proton-pumping, in accordance with the data in Table 1.

This work shows that the four electron transfers from cytochrome *c* to the bimetallic centre (Fig. 1) are non-equivalent with respect to coupling to proton-pumping. The steps reducing the 'peroxy' and 'oxyferryl' intermediates are probably each coupled to translocation of 2 H<sup>+</sup>, whereas the two other electron transfers of the catalytic cycle (Fig. 1b, box II) are not coupled to proton-pumping.

It is generally assumed that each electron is transferred equivalently from cytochrome *c* first to the Cu<sub>A</sub> and haem *a* redox centres, and then to the bimetallic centre. With respect to the latter, however, the four electron transfers are non-equivalent (Fig. 1b), suggesting that neither Cu<sub>A</sub> nor haem *a* can *a priori* have primary mechanistic roles in proton-pumping. Instead, it seems that the bimetallic centre is itself critically involved in the mechanism. There are, however, two alternative possibilities by which either Cu<sub>A</sub> or haem *a* could play a part in proton-pumping without excluding the bimetallic centre. First, it is conceivable that either Cu<sub>A</sub> or haem *a* might specifically carry electrons from cytochrome *c* to P and F, but not to O or H, although there is no experimental support for this at present. Second, there might be some co-operativity between Cu<sub>A</sub> or haem *a* with the bimetallic centre. The latter has been demonstrated, whereas interactions with Cu<sub>A</sub> are small or non-existent<sup>11-14</sup>. Haem *a* could thus have a specific configuration when oxygen is bound to the bimetallic centre (states P and F), and could therefore be mechanistically involved in proton-pumping<sup>15,16</sup>.

The observed 'mechanistic' 2 H<sup>+</sup>/e<sup>-</sup> ratio in itself obviously constrains possible mechanisms of proton-pumping. Interestingly, proton-pumping by bacteriorhodopsin has the same stoichiometry per photocycle. Here the mechanism includes

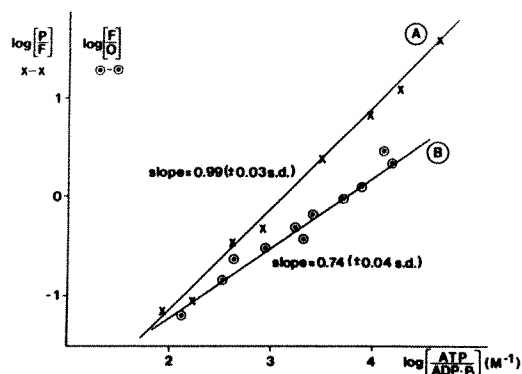


FIG. 2 Dependence of the P/F (A) and F/O (B) equilibria on phosphorylation potential. Rat liver mitochondria (0.77  $\mu$ M cytochrome *aa*<sub>3</sub>) were suspended in 0.2 M sucrose-20 mM KCl-20 mM HEPES-Tris buffer in the presence of 3  $\mu$ M rotenone, 0.25  $\mu$ g ml<sup>-1</sup> antimycin, 5  $\mu$ M myxothiazol, 0.75 mM EGTA and 0.96 mM potassium phosphate. Reaction temperature, 25°. In A, 1.5 mM potassium ferricyanide was also present (pH 8.3). In B, 1.5 mM each of potassium ferricyanide and ferrocyanide were present (pH 7.2). Varying amounts of ADP and ATP were added in different incubations, and the corresponding concentrations of P, F, and O determined by deconvolution<sup>7</sup> of spectroscopic data at 583 and 607 nm, with 630 nm as reference. The straight lines are derived from a linear regression analysis of the data. The slopes (varying between 0.9 and 1 for A and between 0.70 and 0.75 for B in different experiments) indicate the number of ATP molecules hydrolysed per molecular event.

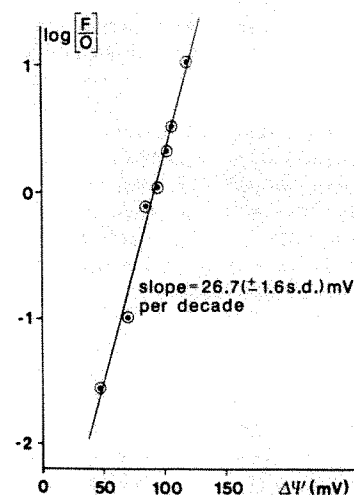


FIG. 3 Dependence of the F/O equilibrium on membrane potential. Rat liver mitochondria were suspended in 0.25 M sucrose-20 mM HEPES-Tris buffer at pH 8.3, in the presence of rotenone, antimycin, myxothiazol and EGTA (see Fig. 2), and 1.5 mM sodium ferricyanide. After 15 s 0.4  $\mu$ g ml<sup>-1</sup> of valinomycin was added, and  $\Delta\psi$  recorded as described for Fig. 2. KCl was added at different concentrations to vary  $\Delta\psi$ . Without added KCl the extramitochondrial K<sup>+</sup> concentration was 1 mM after adding valinomycin (as measured potentiometrically). Much of this K<sup>+</sup> stems from the intramitochondrial space and part stems from the buffer used to isolate the mitochondria. The amounts of F and O were determined at each potential, as described for Fig. 2; in these conditions only negligible amounts of P were formed.  $\Delta\psi$  was estimated from the Nernst equation, assuming 120 mM intramitochondrial K<sup>+</sup>. Linear regression analysis gave the line of best fit shown.

active participation of both the chromophore and acidic amino-acid residues in the apoprotein<sup>17</sup>. Directly coupled mechanisms, which typically involve the redox centres, the substrates and the products<sup>18-21</sup>, are more strongly circumscribed by the H<sup>+</sup>/e<sup>-</sup> ratio of 2 than are indirect mechanisms with essential contributions from the protein structure. This is not only because it is easier, at present, to explicitly describe directly coupled mechanisms chemically, but also because direct coupling of two-proton transfer to one-electron transfer is less common in biological chemistry. Yet, such coupling does exist, as exemplified by the superoxide/hydrogen peroxide redox couple at physiological pH.

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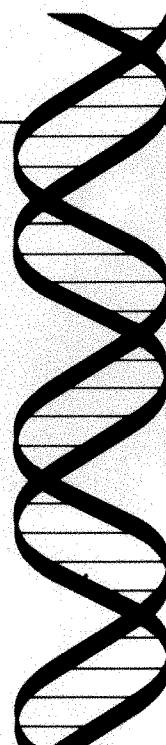
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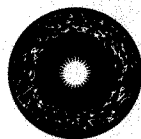
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(W6098)A

## MEMORIAL UNIVERSITY OF NEWFOUNDLAND DEPARTMENT OF BIOCHEMISTRY PHYSICAL BIOCHEMISTRY

The Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, invites applications from qualified individuals for a tenure-track faculty position effective October 1, 1989. Applicants should have an interest in research and teaching in physical biochemistry or protein structural chemistry or both. Applicants should have a Ph.D. and productive postdoctoral experience if appointed at the Assistant Professor rank, and a proven record of independent research if appointed at the Associate Professor or Professor rank. The successful candidate will be required to establish a strong independent research program and to undertake undergraduate and graduate teaching. Applicants should submit a *curriculum vitae* and names of three referees to **Dr. K.M.W. Keough, Head, Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9**, before August 1, 1989.

In accordance with Canadian Immigration policy, consideration, in the first instance, will be given to Canadian citizens and permanent residents of Canada. Others are encouraged to apply and will be considered. (NW3660)A

## UNIVERSITY OF WATERLOO GROUNDWATER RESEARCH

The Waterloo Centre for Groundwater Research (a Centre of Excellence funded by the Government of Ontario) is seeking applications for a research position in Environmental Biochemistry/Microbiology.

The appointment will be at the University of Waterloo at a professorial rank dependent upon qualifications and experience for a term of five years contingent upon continued funding. The successful applicant will work as part of a multidisciplinary team involved in research on aspects of groundwater contamination and will develop a research program with major support obtained from industry and government agencies. A candidate with a Ph.D. degree in an area of biochemistry or microbiology with applications to biochemical studies of biodegradation and biotransformation of organic compounds in groundwater will be given preference.

The closing date for applications is upon appointment of a suitable candidate. The appointment will be effective July 1st 1989 or as soon as possible after that date. With permission of Employment and Immigration Canada, competition is open to Canadian citizens, permanent residents and non-Canadians. Please send a complete *curriculum vitae* and the names, addresses and telephone numbers of three referees to **D.J.A. Smyth, Manager, Waterloo Centre for Groundwater Research, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.** (NW3642)A

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(9133)A

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Solar and Stellar Seismology (Professor G R Isaak)	--ref PH4312
Environmental/Biomedical Physics (Dr T D Beyon)	--ref PH4313
Chemical Physics (Professor D Smith, FRS)	--ref PH4314

These research areas are being accorded high priority by the School and University because of notable recent advances made in them at Birmingham and because of their considerable potential for future development. It is hoped that the persons appointed will take up their posts on or before 1 October 1989. The salary will be in the Lecturer A/B range (£9,260-£19,310, subject to review) plus superannuation.

For further particulars of the above posts and an application form, telephone 021-414 6383 quoting the appropriate reference number. Applications (3 copies) should be sent to the **Senior Assistant Registrar, Faculty of Science, PO Box 363, Birmingham B15 2TT** by 31 May 1989.

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For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the **Personnel Office, Senate House, Bristol BS8 1TH**. Please quote reference A356.

The closing date is 12th May 1989.

(9075)A

## CHIEF, LABORATORY OF MOLECULAR GENETICS

### NATIONAL INSTITUTES OF HEALTH

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#### DEPARTMENT OF HEALTH AND HUMAN SERVICES

The Intramural Research Program of the National Institute on Aging (NIA), Baltimore, Maryland invites nominations and applications for the position of Chief, Laboratory of Molecular Genetics. This is a Civil Service Position with a salary range of \$57,158 to \$74,303 per annum depending upon qualifications. Candidates may be eligible for Physician's Comparability Allowance up to \$20,000 per year. Alternatively, candidates may be eligible for appointment in the Commissioned Corps of the U.S. Public Health Service.

The Chief of the Laboratory of Molecular Genetics is responsible for planning, directing and conducting research programs which use molecular and genetic approaches to the study of aging and related processes; for recruiting staff and providing professional leadership for senior staff in their projects; and for originating, designing, and conducting independent and collaborative research on age and disease related alterations in the structure and regulation of genes.

Applicants must have a Doctoral degree with a record of outstanding scientific accomplishment in the field of Molecular Genetics. In addition, applicants should possess qualifications that demonstrate competence in executive level management and leadership ability.

Further information may be obtained by contacting Ms. Barbara Hughes at (301) 550-1733. An Application for Federal Employment (SF-171) with a current curriculum vitae and bibliography should be submitted to:

**Personnel Office, Gerontology  
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National Institute on Aging  
4940 Eastern Avenue  
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Agreement was recently reached between Murdoch University and The University of Western Australia to seek a merger of the two institutions by January 1990.

Applicants, who may belong to any area of plant sciences, are expected to have an international research reputation including a strong record of external research support, a proven ability for leadership and a commitment to teaching. (Ref: 0010)

**SALARY:** \$A63,919 per annum.

Conditions of appointment include superannuation, long service leave, access to outside studies programmes, payment of fares to Perth for appointee and dependant family, removal and settling-in allowances.

There is no prescribed application form. Applicants are invited to submit a full curriculum vitae supported by an appropriate letter including the names and addresses of three referees.

Applications and requests for further information should be sent to:

**The Chief Personnel Officer  
Murdoch University  
MURDOCH WESTERN AUSTRALIA 6150**

**Telephone: (619) 332 2283**

Applications will close on 2 June 1989.

Applicants resident in the United Kingdom, Europe or Africa, may obtain supporting statements from and should lodge one copy of any application with:

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(W6086)A

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The *research technician* (grade 3) will be responsible for the purification and assay of DNA binding proteins; experience in microbiology and/or protein purification would be an advantage. Applicants should have at least 3 years relevant experience and minimum qualification of OTEC or equivalent. Applications will also be considered from recent graduates for appointment as graduate Trainees for an initial 12 months.

All three positions are available for up to three years. Salaries will be in the range £9,865-£15,720 p.a. for the postdoctoral positions and £6,636-£7,828 p.a. for the technician. Graduate Trainees will receive an initial £6,395 p.a. (all salary scales under review). Applicants should send a C.V. including names and addresses of two referees to **Professor G. Roberts, Department of Biochemistry, University of Leicester, Leicester LE1 7RH** by [closing date to be inserted four weeks after insertion of advert]. For further information contact Dr. Eva Hyde or Dr. Vasudevan Ramesh (0533 523051) or Professor Gordon Roberts (0533 523054). (9106)A

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Further information may be obtained from: **Dr P A Whittaker, University Department of Clinical Biochemistry, Southampton General Hospital, Tremona Road, Southampton SO9 4XY. (Telephone (0703) 777222 Ext 3856).** To whom applications with a full CV and names of two referees should be submitted by 18th May 1989. (9127)A



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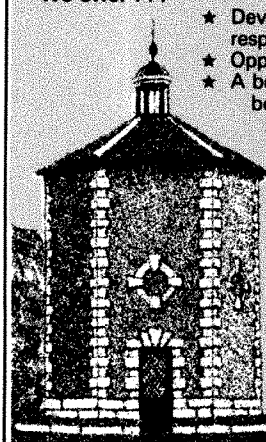
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For further details and application form, please contact the **Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE** (Tel. 01-672 9944 x56020), quoting reference 53/89. Informal enquiries to Dr D Bennett (x55202). Closing date 25 May 1989. (9093)A

**The Elmer V. McCollum Professor  
and  
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The Johns Hopkins University  
School of Hygiene and Public Health**

Applications and nominations are invited for the position of Chairperson of the Department of Biochemistry at the Johns Hopkins School of Hygiene and Public Health. The general research interests of the current faculty include: Biochemistry — enzyme structure and mechanisms; peptide chemistry; chromatin and nucleic acid chemistry; mechanisms of nucleic acid replication, recombination and repair; structure and function of cell surface molecules; somatic mutation and antibody diversity; cell metabolism; influence of nutrition in carcinogenesis. Biophysics — structure and interaction of biopolymers in cells; synthesis of oligo- and polynucleotides of designed structure and sequence; structure and function of the mammalian genetic apparatus; development of antiviral and chemotherapeutic agents; basic mechanisms of aging. The Department also participates in a University-wide concerted effort in areas of macromolecular assemblies and interactions for which it has received considerable Federal and private support.

Candidates must have an outstanding record of research achievement in any area of biochemistry, biophysics or molecular biology, whether or not the area is currently represented in the Department; and the desire and ability to provide strong leadership to an already excellent, vibrant, growing department in the interdisciplinary setting of the School of Hygiene and Public Health.

Interested persons are encouraged to submit a curriculum vitae to:

**Dr. Barry Zirkin**  
**Chairman of the Biochemistry Search Committee**  
**Division of Reproductive Biology**  
**The Johns Hopkins School of Hygiene and Public Health**  
**615 North Wolfe Street**  
**Baltimore, MD 21205**

The Johns Hopkins University is an Equal Opportunity employer

(NW3650)A

**THE JOHN THOMAS LADUE MCGINTY  
EMINANT SCHOLAR CHAIR  
in Marine Biology  
at Florida Atlantic University,  
a Member of the Florida State University System**

Applications and nominations are invited for the John Thomas Ladue McGinty Eminent Scholar Chair in Marine Biology at Florida Atlantic University. This position is supported by a \$1,000,000 endowment and will be filled at a senior level in the Department of Biological Sciences, College of Science.

Applicants and nominees should have a distinguished academic and research reputation that includes a successful record in obtaining contract and grant support. The primary responsibilities of the successful candidate will be to contribute significantly to the expansion of the teaching and research programs in the Department of Biological Sciences and to provide graduate level training in his or her field of speciality.

Florida Atlantic University is a four-year and graduate state university with an enrollment of over 11,000 students and a plan for growth in numbers of students and in graduate programs over the next five years. The Florida Board of Regents has initiated seven new doctoral programs in Science and Engineering at Florida Atlantic University in recent years. In addition, the University currently has nine Eminent Scholar Chairs, and funding for a tenth has been pledged. The combination of this program development and of the impact of nine Eminent Scholar Chairs has brought about a quality research and teaching environment on campus that will be significantly enhanced by the addition of the John Thomas Ladue McGinty Scholar.

The central campus of Florida Atlantic University is located in Boca Raton, approximately two miles west of the Atlantic Ocean and forty miles north of Miami. The Department of Biological Sciences is housed in a five-storey building devoted primarily to teaching and research in biology. An oceanside marine laboratory will soon be under construction, and is located within a ten-minute drive of the campus. It will feature a flow-through seawater system, wet tables, and culture ponds.

The Eminent Scholar Search Committee represents faculty, students, and the community. The deadline for applications is July 1, 1989. Applications and nominations should be addressed to: **Dr. Walter R. Courtenay, Jr., Chair, Eminent Scholar Search Committee, Department of Biological Sciences, Florida Atlantic University, Boca Raton, Florida 33431-0991.**

An Affirmative Action/Equal Opportunity Institution

(NW3635)A

**NATIONAL INSTITUTES  
OF HEALTH**

**PUBLIC HEALTH SERVICE**

**DEPARTMENT OF  
HEALTH AND HUMAN  
SERVICES**

The National Institutes of Health (NIH) invites nominations and applications for the position of Director, Office of Scientific Integrity. This is a Civil Service position in the Senior Executive Service (SES), with a salary range of \$68,700 to \$76,400 per annum. The person selected may be eligible for Physician's Comparability Allowance ranging from \$5,000 to \$20,000 per year. A one year probationary period must be served by the individual selected if not currently in the Senior Executive Service.

The Office of Scientific Integrity is responsible for detecting, investigating, reporting and resolving allegations of scientific misconduct.

The Director of the Office of Scientific Integrity is responsible for developing and implementing policies and procedures relating to scientific misconduct in extramural and intramural research activities.

For more information contact Ms. Dolores Jeter at (301) 496-6521. The following forms are required:

Application for Federal Employment (SF-171) accompanied by a current curriculum vitae and bibliography.

These must be submitted to:

**Ms. Dolores Jeter**  
**National Institutes of Health**  
**Office of the Director,**  
**Personnel**  
**Building 31,**  
**Room 1C-23**  
**9000 Rockville Pike**  
**Bethesda, Maryland 20892**



**APPLICATIONS MUST BE  
POSTMARKED NO LATER THAN  
MAY 23, 1989.**

All qualified candidates will receive consideration without regard to race, religion, color, sex, age, national origin, lawful political affiliation, marital status, union membership, or nondisqualifying physical or mental handicap.

NIH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW3634)A

# VIROLOGIST

**P**fizer is one of the world's leading research-based pharmaceutical companies. Our Sandwich research facility, where a significant part of our anti-infective drug discovery programme is based, currently employs about 700 scientists and support staff. Our present anti-viral strategies include both classical and molecular genetic-based approaches to viral diseases including AIDS. We are seeking to expand the virology group and require a Post-doctoral Virologist to join our team.

**C**andidates should have:

- A PhD in Virology
- 1-3 years Post-doctoral experience in Virology or Molecular Genetics of viruses

**We** can offer:

- The chance to develop within a dynamic anti-viral team
- A highly competitive salary with merit-related bonuses of up to 20% of salary

- Generous relocation assistance and other benefits including pension, life assurance and private health care schemes
- Flexible working hours and an active sports and social club

**T**his is a particularly exciting time to join Pfizer as we have a multi-million pound expansion of our research facilities under construction, demonstrating our long-term commitment to research and enabling our scientists to look forward with confidence to the 1990's and beyond.

**We** are located within easy reach of both Canterbury and the Continent and there is a wide range of housing with prices amongst the lowest in the south-east.

**I**f this advertisement attracts your interest, please write in confidence, giving full details of career-to-date to Mr D O'Callaghan, Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ. Tel: (0304) 616415.



(9100)A

## YALE UNIVERSITY SCHOOL OF MEDICINE

### Postdoctoral Positions in DEVELOPMENTAL NEUROBIOLOGY

Two positions are available to study molecular and cellular aspects of development in the mammalian visual system.

One position will study the regulation of expression of identified retinal genes in order to understand how known cellular events in retinal development lead to changes in expression of groups of genes (see Mol. Cell. Biol. 8:1570, 1988). Good background in molecular biology is preferred.

The other position will use monoclonal antibodies and biochemical methods to study the differentiation of cell types and the formation of synaptic circuits in visual cortex (see TINS 12:28, 1989). A good background in immunocytochemical methods is preferred.

Send C.V. and names of two referees to: **Dr. Colin J. Barnstaple, Department of Ophthalmology and Visual Science, Yale University School of Medicine, P.O. Box 3333, New Haven, CT 06510.**

(NW3653)A

## UNIVERSITY OF ABERDEEN DEPARTMENT OF ZOOLOGY

### POSTDOCTORAL RESEARCH ASSISTANT

Candidates are invited to apply for the above position, which is available for a three year period, to investigate how certain strains of fish bacterial pathogens avoid killing by salmonid macrophages. Preference will be given to candidates with immunological and tissue culture experience; previous experience with fish cells is not vital. For further details on the project contact Dr C Secombes (tel 0224 272872) or Mrs Reid (tel 0224 272859) in the Department of Zoology. Starting salary up to £10,460 on the IA (postdoctoral) Scale.

Further particulars and application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** (tel 0224 273500) to whom applications (2 copies) should be returned by 19 May 1989 quoting reference number LW/020. (9074)A

## UNIVERSITY OF LONDON

### Chair of Clinical Veterinary Medicine

at

### The Royal Veterinary College

The Senate invites applications for the established Chair of Clinical Veterinary Medicine at The Royal Veterinary College.

Candidates should have a proven record of excellence and leadership in research and clinical work. The College would be especially pleased to receive applications from those whose interest is in farm animals.

Potential applicants are encouraged to contact the Principal of The Royal Veterinary College, Professor Lance Lanyon, telephone: 01-387 2898, fax: 01-388 2342.

Intending applicants should obtain further particulars from the **Academic Registrar, (N) University of London, Senate House, Malet Street, London WC1E 7HU**, before submitting applications (10 copies).

Closing date for applications: 15 June 1989 (9129)A

## Ph.D.'s

Biotech Research Labs in Rockville, has openings for 2 Ph.D.'s.

**HIV RESEARCH:** Individual will be responsible for HIV research. We are looking for an individual who enjoys a challenge to help solve the mysteries of HIV.

**MOLECULAR BIOLOGIST:** Individual must have experience with recombinant DNA techniques. Familiarity with characterization of genomic DNA in construction of expression vectors is preferred.

We offer competitive salaries and an excellent benefits package. If interested, please submit a CV to:

### BIOTECH RESEARCH LABS

1600 East Gude Drive, Rockville, Maryland 20850. Attn: Personnel  
EOE M/F/H/V (NW3646)A



**INSTITUTE OF HYDROLOGY  
WALLINGFORD, OXON**

## **HYDROGEOLOGIST**

We are seeking an experienced, innovative, numerate hydrogeologist to join the Consulting Services Section of the Institute of Hydrology. This is a multi-disciplinary team which provides a hydrological and hydrogeological advisory service to overseas governments and British consultants.

The work is directed primarily towards water resources studies in developing countries and, although based at Wallingford, the successful candidate will be expected to travel overseas, mainly on short-term assignments. The hydrogeologist would also be concerned with ground-water pollution and environmental impact studies, often for shallow alluvial aquifers in the UK.

The successful candidate must be highly motivated and must be prepared on occasions to work with minimal supervision or support. Applicants must be skilled in modern mathematical and statistical techniques and must be competent with both micro and mainframe computers. An ability to programme in FORTRAN is essential as support to ongoing software development would form one part of the job. Ideally applicants would have a relevant postgraduate qualification.

The appointment is for three years. Salary will be in the range of £10,026 per annum to £13,460 per annum, or £12,445 per annum to £17,032 per annum depending on age, qualifications and experience. At the higher salary level overseas experience or project management experience would be expected.

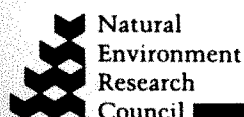
Generous holidays and non-contributory pension scheme.

The Institute of Hydrology is a component body of the Natural Environment Research Council.

NERC is an Equal Opportunities Employer.

Further information and application forms may be obtained from **Mrs S Fenton, Institute of Hydrology, Wallingford, Oxon OX10 8BB. Telephone 0491-38800.**

Closing date for applications is 18 May 1989.



Natural  
Environment  
Research  
Council

(9087)A

**UNIVERSITY OF ALBERTA, DEPARTMENT OF GENETICS**

## **ASSISTANT PROFESSOR**

Applications are invited for a tenure-track position in the Department of Genetics. We are seeking a yeast molecular geneticist, although excellent candidates in other areas of genetics will be considered seriously. Candidates should have a Ph.D. and relevant post-doctoral research experience. The appointee will establish a vigorous independent research program and teach basic genetics as well as advanced molecular genetics. The appointment would commence September 1, 1989 or January 1, 1990 or thereafter. Salary will be commensurate with experience; the Assistant Professor range is currently \$33,144 - \$47,280.

Candidates should send a curriculum vitae, reprints of several research publications, a one-page statement of future research interests, and the names and addresses of three academic referees to:

**Dr. Frank E. Nargang; Chairman, Search Committee; Department of Genetics; University of Alberta; Edmonton, Alberta, T6G 2E9; Canada.**

The closing date for applications is June 30, 1989.

In accordance with Canadian Immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada. The University of Alberta is committed to the principle of equality in employment. (NW3658)A

UNIVERSITY OF

# **essex**

Department of Biology

## **Effluent Treatment by Anaerobic Digestion**

A Research Officer is required to carry out laboratory investigations of a novel anaerobic treatment process for organic effluents, including land fill leachate. Applicants should have some microbiological expertise, and experience with anaerobes would be advantageous. The Research Officer will preferably be at postdoctoral level on Grade IA (£9,865-£15,720 per annum), but well qualified postgraduates will also be considered for appointment on Grade IB (£8,675-£11,680 per annum). The appointment is for 2 years in the first instance, with the possibility of extension for a further year. Informal enquiries may be made to Dr D.B. Nedwell (0206 872211).

**Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (R/874/N), University of Essex, Wivenhoe Park, Colchester, CO4 3SQ by 12th May 1989. Further particulars of this post may be obtained by telephoning Colchester (0206) 872462 (24 hours).**

(9102)A

**UNIVERSITY OF LEICESTER**

**X-ray Astronomy Group, Department of Physics**

## **Support Astronomer — ROSAT Guest Observer Programme**

Applications are invited for a UGC-funded, post-doctoral position in support of the UK ROSAT Guest Observer Programme. The ROSAT project is an international collaboration between W. Germany, the UK and the USA. The satellite, due for launch in February 1990, will carry out the first imaging all-sky survey in the XUV and soft X-ray bands. Subsequent pointed observations of individual targets will be made through guest observer programmes in each country. The Guest Observer Support Astronomer will play a leading role in running the UK Guest Observer Centre, which is to be based at the University of Leicester. He/she will have overall responsibility for assisting visiting guest observers with the analysis of their ROSAT observations. The Centre will provide dedicated computing facilities for Guest Observers based on VAX workstations clustered with the Leicester Starlink node computers.

We are looking for a post-doctoral scientist with a background in observational astronomy and experience of data analysis systems who is also keen to develop an astrophysics research programme based on ROSAT. There may also be an opportunity to contribute to the ROSAT data analysis software. The appointment will be for three years, starting in September 1989 or soon after. The salary will be on the R & A IA scale, range £9,865-£15,720 (under review) depending on age and experience.

Applications (together with a curriculum vitae and the names of two referees) should be sent to **Professor K.A. Pounds, Department of Physics and Astronomy, University of Leicester, Leicester LE1 7RH, by 18 May 1989.** (9111)A

**ROYAL FREE HOSPITAL SCHOOL OF MEDICINE**  
(University of London)

Department of Neurological Science

## **POST-DOCTORAL RESEARCH FELLOW/BIOCHEMIST MITOCHONDRIAL DISEASES**

Applications are invited from post-doctoral biochemists to work on the development and tissue specificity of the mitochondrial respiratory chain proteins and their relationship to human mitochondrial diseases. Experience in protein purification immunochemical techniques and enzyme assays would be an advantage. This project, funded for three years by Action Research, will involve collaboration with the Department of Biochemistry, St Bartholomew's Hospital Medical College and the Institute of Neurology, London.

Salary on Range 1A: £9,865-£15,720 p.a. plus £1,650 London Allowance, according to age and experience.

Informal enquiries to Dr Mark Cooper. Tel: (01) 253-9237. Full job description available from the **School Office, R.F.H.S.M., Rowland Hill Street, London, NW3 2PF.** (Tel: 01-794-0500 Ext: 4262), to which applications (two copies of curriculum vitae with names and addresses of two referees) should be sent by **12 May 1989.** Please quote Ref: **RF/NS.** (9090)A



UNIVERSITY COLLEGE LONDON

## Departments of Pharmacology and Clinical Pharmacology

These Departments have a number of Research Council Studentships to offer, for work in the following areas:

- 1) Synaptic, receptor and ion channel pharmacology (D.A. Brown, D. Colquhoun, FRS, D.H. Jenkinson and D.G. Haylett).
- 2) Cell biology (cell to cell communication) in the context of toxicology (A.E. McLean).
- 3) Immunopharmacology: role of mediators and second messengers (M.M. Dale, J.C. Foreman).

Applicants should hold, or expect to obtain, an upper second or better in a relevant biological science, and should be able to comply with the residence requirements of the MRC and SERC.

Please write in the first instance to the **Departmental Postgraduate Tutor (Professor D.H. Jenkinson, Department of Pharmacology, University College London, Gower Street, London, WC1E 6BT)** enclosing a full C.V. giving an indication of your interests, and the names of two academic referees. (9101)A

## ST MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, Technology and Medicine)  
(University of London)

Norfolk Place, London W2 1PG

## LECTURER IN ANATOMY

Applications are invited for a Lectureship (available from October 1989) in the Department of Anatomy and Cell Biology (within the Division of Basic Medical Sciences). Candidates should have a record of active research in the biomedical sciences and must be able to make a major contribution to one or more of the Department's main teaching areas (Human Anatomy, Cell Biology and Histology, Developmental Biology and Neuroanatomy).

The Department's current research includes grant-funded projects on CNS Responses to Injury, Alzheimer's Disease, Cell Interactions in Peripheral Nerves, Mechanisms of Cell Movement, and Transport and Permeability in Epithelia and Endothelia. Facilities are particularly good for light and electron histochemical methods and for quantitative microscopy. A research interest in the central nervous system is preferred, but candidates able to contribute to existing strengths or to develop new ones will be considered on merit.

Prospective applicants are invited to 'phone the Head of Department, Professor Tony Firth ((01) 723-1252 Ext 5343).

Applications (6 copies) in form of full C.V. with names and addresses of 2 referees should be sent to Personnel Department at above address by 2 June 1989, from where further written details should first be obtained. (9094)A

## UNIVERSITY OF OXFORD



### SIR WILLIAM DUNN SCHOOL OF PATHOLOGY Research Assistant 1A

Salary £9865-£15,720 (under review)

A post-doctoral position is available for three years for research on the role of the nucleoskeleton in transcription and replication. (See J. Cell Sci. 90, 1-6, 1988 for a review). The position is funded by the Cancer Research Campaign from April 1989, but could start later by arrangement. Further details may be obtained from Dr. Peter Cook (Tel: 0865-275528).

Applications in writing, together with c.v. and names and addresses of two referees should be sent to **The Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE.**

Closing date for applications 19 May 1989.

(9112)A

*The University is an Equal Opportunity Employer*

## School of Biology and Biochemistry and The Institute for Animal Health, Pirbright



## RESEARCH ASSISTANT IN MOLECULAR VIROLOGY

The above post is supported under the LINK Project Scheme (QUB-AFRC) for two years to work on the construction of chimeric bovine enterovirus (BEV) and Foot and Mouth Disease strains for future vaccine development. The successful applicant will work principally at the Institute of Animal Health and be in close collaboration with staff in Queen's where vector development work is currently underway.

Applicants must have or expect to obtain an Honours degree in a biological science related subject and preferably have some experience in molecular biology.

Commencing salary in the range £8,676-£9,867 (under review) with eligibility for USS.

Informal enquiries may be directed to Professor S. J. Martin (Belfast (0232) 329241, ext 2491) or Dr. A. M. King (Pirbright (0483) 232441).

Applicants, quoting ref. 89/AA, should submit a curriculum vitae, including the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN.

Closing date: 30 May 1989.

The University is an Equal Opportunity employer. (9123)A



The Queen's University of Belfast.



## ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

DEPARTMENT OF VIROLOGY

### TWO POSTDOCTORAL SCIENTISTS ONE RESEARCH ASSISTANT

required to join expanding team investigating cytomegalovirus infections. Each post is for 3 years.

**POSTDOCTORAL POSITION ONE (ref. PD/RF)** will modulate the cell surface expression of Class I HLA molecules to determine if CMV can use these proteins as cellular receptors. Experience of recombinant DNA or immunological techniques desirable.

**POSTDOCTORAL POSITION TWO (ref. JG/RF)** will study the interaction between CMV and  $\beta_2$ -microglobulin to characterise, sequence, clone and express the viral proteins involved. Experience of the purification of proteins (not necessarily viral) from a complex mixture would be an asset.

**RESEARCH ASSISTANT (ref. SB/RA)** will participate in the studies of CMV,  $\beta_2$ -microglobulin and Class I interactions. Immunological experience desirable. Salary will be paid on Research Scales 1A/1B as appropriate plus £1,650 London Allowance. The Department is situated in modern purpose-built accommodation and is well equipped. These projects will provide ideal opportunities to broaden experience of modern virological techniques. Informal visits are welcome (tel. 01-794 0500 x3210 (Dr. Griffiths), x4118 (Dr. Grundy) or x4201 (Dr. Baldwin)).

Further particulars can be obtained from the **SCHOOL OFFICE (x4262), R.F.H.S.M., Rowland Hill Street, London NW3 2PF**, to which applications (FOUR copies of curriculum vitae including the names and addresses of two referees) should be sent by 12 May. (9099)A



# SCIENCE IN EUROPE



## University of Amsterdam

The Faculty of Biology has a vacancy for an

### assistant professor (m/f)\* N.6768.

in the molecular biology of lower plants  
Full-time

The position is within the department of molecular cell biology.

Applications are invited for a five-year appointment to augment molecular biological approaches in the program "Sexual development in the green alga *Chlamydomonas*", in the Section Plant Physiology.

Requirements and qualifications:

- satisfactory didactic qualities for participating in advanced undergraduate and graduate courses
- postdoctoral experience in any area of plant molecular biology
- considerable organizing ability
- supervising PhD and MSc students and initiate novel approaches in the above mentioned program.

\* Women in particular are invited to apply.

The successful applicant will be expected to learn the Dutch language within two years.

The gross salary depends on experience and ranges from Dfl. 6.957,- to Dfl. 9.957,- per month (Dutch Civil Servants Code).

Further information can be obtained by telephoning or writing to the secretary of the nomination committee, Prof. H. van den Ende, Department of Molecular Cell Biology, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands (telephone 031 - 20 525 7847) to whom applications with a curriculum vitae - within 3 weeks - should also be sent. A statement of research interests and the names of two referees should be forwarded.

(W6066)A

## PROFESSOR OF ASTROPHYSICS WITH COSMOLOGY\*) AT STOCKHOLM OBSERVATORY

The University of Stockholm hereby announces a position as professor of astrophysics with cosmology at Stockholm Observatory open for application. The successful candidate will be expected to be actively engaged in research, to participate in teaching (in Swedish or English) including supervision of graduate students, and to take part in local, national, and international administration duties of the Observatory.

\*)The area of responsibility for the position covers the whole of astrophysics including cosmology. The candidate is required to have a scientific background within *either one* of these areas. The specialization can be observational or theoretical.

Application: The application should be made to "The Swedish Government" and the mailing address is:

**Rektorsämbetet  
Stockholm University  
S-106 91 STOCKHOLM  
Sweden**

The application should be accompanied by:

1. Detailed curriculum vitae and bibliography
2. Short written account of scientific work and teaching experience
3. Copies of records or documents that the applicant wishes to submit to verify his qualifications
4. Four reprints or preprints of each of the applicant's scientific publications numbered according to the publication list

Closing date: The formal application and documents under item 1 above must be received by the University not later than **May 8, 1989**. (It is advisable to submit the formal application by telex to 8105199 UNIVERS or telefax (0)8-159522.)

Documents under items 2-4 above must be received by the University not later than **May 29, 1989**. (W6092)A

## Ausschreibungstext zur Planstelle eines/einer Ordentlichen Universitätsprofessors/Universitätsprofessorin für Pharmazeutische Chemie (Nachfolge Univ.-Prof. Dr. Wilhelm KLÖTZER)

Am Institut für Organische und Pharmazeutische Chemie der Naturwissenschaftlichen Fakultät der Universität Innsbruck ist die Planstelle eines/einer

## Ordentlichen Universitätsprofessors/Universitätsprofessorin für Pharmazeutische Chemie (Nachfolge Univ.-Prof. Dr. W. KLÖTZER)

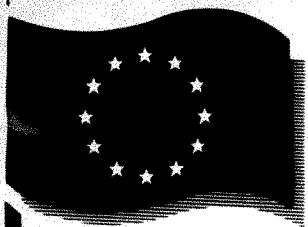
zu besetzen. Der/die Stelleninhaber/in hat das Fach Pharmazeutische Chemie in Forschung und Lehre zu vertreten. In diesem Fach sollte er/sie sowohl in der Forschung entsprechend ausgewiesen sein als auch Erfahrung in der Lehre nachweisen können.

Bewerber/innen mit einschlägiger Habilitation oder gleichwertiger Qualifikation werden gebeten, ihre Unterlagen (Lebenslauf, Schriftenverzeichnis, Sonderdrucke der fünf wichtigsten Publikationen, Angaben über Erfahrung in der Lehre) bis **31. Juli 1989** beim Dekan der Naturwissenschaftlichen Fakultät der Universität Innsbruck, A-6020 Innsbruck, Innrain 52, einzureichen.

Die Universität Innsbruck strebt eine Erhöhung des Anteils von Frauen am wissenschaftlichen Personal an und fordert deshalb qualifizierte Frauen nachdrücklich auf, sich zu bewerben.

(W6096)A





## THE COMMISSION OF THE EUROPEAN COMMUNITIES

is carrying out a selection procedure with a view to drawing up a reserve list from which to appoint staff in grade A3 for implementing the

### FUSION PROGRAMME

(COM / R / A / 37) HEAD OF UNIT / ADVISER (grade A3)

**Duties:** under the authority of the Director of the Fusion Programme, either:

☐ heading the unit responsible for coordinating Community research on the physics of fusion and for scientific and technical management of the Fusion Associations; ☐ or coordinating the work involved in drawing up the Community's Fusion Programme and acting as its representative to other Community institutions (European Parliament, Council) and outside bodies.

**Qualifications:** University education with degree or diploma in a relevant discipline.

**Experience:** at least 15 years' work in controlled thermonuclear fusion or an associated field.

#### GENERAL CONDITIONS

**Contracts:** only temporary contracts of fixed or indefinite duration are offered to research staff. **Nationality:** candidates must be a national of one of the Community Member States. **Place of employment:** Commission staff must be available to work in any of the Commission's places of activity and in many cases outside their country of origin. **Age:** candidates must have been born after 1 June 1933. **Knowledge of languages:** candidates must have a thorough knowledge of one Community language (Danish, Dutch, English, French, German, Greek, Italian, Portuguese or Spanish) and a satisfactory knowledge of a second Community language.

**Closing date:** requests for application forms must be made in writing not later than 15.5.89 (postmark) to the following address: C.E.C., Secretariat for Selection Committees Research, SDME R2 / 82, rue Montoyer 75, B-1040 Brussels (tel.: 02 / 235.56.60).

These application forms, duly completed and signed, must reach the above address not later than 6.6.1989.

**Eligibility:** candidates must ensure that: ☐ the above deadlines are met; ☐ their application form is legible, complete and signed; ☐ copies of certificates and other documents specified in the application form are attached; ☐ the other conditions set out above are satisfied, failing which they will be disqualified.

The Commission is an equal opportunities employer.

(W6071)A

### UNITE DE NEUROBIOLOGIE INSERM U6 MARSEILLE POSTDOCTORAL RESEARCH FELLOW IN NEUROBIOLOGY

A postdoctoral fellow is required to join a multidisciplinary team concerned with the information processing by the dendrites of mammalian neurones studied in vivo, in brain slices and in culture. The successful applicant will participate in studies on characterisation of receptor-channels with particular emphasis on patch-clamp and optical recording methods. Preference will be given to individuals with a strong research record in biophysical approach to study membrane excitability and with experience in cell culture.

The position is tenable for a period of one year with possible renewal for a second year. The starting date is not later than September 1989. Send curriculum-vitae, a statement of research interests and three letters of reference to:

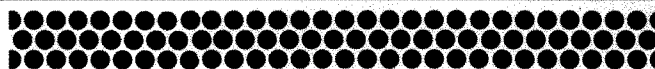
Dr. S. TYC-DUMONT,  
Unité de Neurobiologie, INSERM U6,  
280 Bd Sainte-Marguerite,  
13009 Marseille, France  
(Tel. 91 75 02 00).

(W6090)A

### University of Göttingen, Germany Biochemist, Ph.D.

A postdoctoral position is available immediately for two years with payment according to BAT. Experience with DNA and micro-computers is essential, knowledge of immunology and publication procedures desirable. The appointed person will join a young group dealing with various aspects of genetic diseases and is expected to develop new techniques in this area. Candidates from the U.K. are especially encouraged to apply. Informative documents to Dr. J. Reiss, Institute of Human Genetics, Gosslerstrasse 12d, D-3400 Göttingen.

(W6107)A



The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, West Germany, has the following vacancy:

### TECHNICAL ASSISTANT

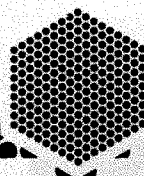
The successful candidate will work in a group concerned with the problems of membrane protein traffic in animal cells. The techniques employed include cell fractionation, in-vitro protein synthesis, DNA/RNA isolation and characterization, site directed mutagenesis. Knowledge of nucleic acid methodology would be advantageous.

Appropriate training and experience in biochemical and molecular biology is essential. Preference will be given to candidates having experience in the application of cell and molecular biological techniques. (DNA manipulations, transfection protein isolation and characterization). A working knowledge of English is essential; a second language (French or German) is desirable.

We offer an above-average salary, plus certain allowances depending on personal circumstances.

Please write briefly for an application form, quoting reference no 88/42, to:

EMBL, Personnel Section,  
Postfach 10.2209, D-6900  
Heidelberg, Federal  
Republic of Germany.



EMBL

(W6093)A

# HOLphar

subsidiary of Fournier Laboratories, a leading pharmaceutical company with a strong commitment to cardiovascular research, is currently seeking two Peptide Chemists for its up-to-date facility in Saarland, West-Germany.

The company offers the opportunity to participate in the creation of a research unit for the study of membrane proteins and lipid-peptide interactions with the aim of designing therapeutic agents effective in the treatment of cardiovascular disease.

Successful candidates will develop new peptide synthesis and peptide modification protocols.

Applicants should have a PhD (or equivalent) as well as in depth knowledge of tBOC and Fmoc chemistries, cleavage chemistries and purification methods. Experience in computer science and peptide modeling is an advantage. Ideal candidates have well developed practical skills and an innovative and enthusiastic approach to work. Knowledge of German is a plus.

All written applications should outline qualifications: educational background and working experience. Send résumé together with three letters of reference to the **Director, Department of Human Resources, HOLphar Arzneimittel GmbH, Justus-von-Liebig-Str. 16, 6603 Sulzbach, West-Germany.**

(W6104)A

## EMBL

The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, West Germany has the following vacancies at its outstation in Grenoble:

### Structural Molecular Biologist

The Grenoble Outstation of the EMBL is a structural molecular biology laboratory which aims to combine expertise in biophysical techniques (notably neutron and X-ray methods) with modern methods of molecular biology. The laboratory is situated at the Institut Laue-Langevin, a high flux neutron source and adjacent to the future European Synchrotron Radiation Facility and is well equipped for neutron scattering, X-ray crystallography (including access to a FAST detector), electron microscopy, dynamic light scattering and molecular biology. Current interests include the molecular biology and X-ray crystallography of surface viral glycoproteins (adenoviruses and myxoviruses) and amino acyl-tRNA synthetases, the dynamics of proteins studied by inelastic neutron scattering, the structure and dynamics of supercoiled DNA and in-vivo deuteration. There are vacancies for up to three staff scientists to contribute to existing projects or to initiate new projects suited to the scientific environment of the laboratory.

Candidates in the following areas will be considered (the examples are not to be considered exhaustive):

- Neutron scattering in biology (e.g. high resolution neutron crystallography of deuterated proteins, or low resolution crystallography of macromolecular complexes).
- X-ray protein crystallography (including new techniques such as diffuse scattering or Laue diffraction).
- Molecular biologist/biochemist with an interest in structural problems (e.g. protein/nucleic-acid interactions, virus/cell interactions).

Staff scientists will be appointed on an initial contract of three to five years on grades 8 or 9 depending on age and experience. An above-average tax-free salary is offered. Certain allowances are payable in addition, depending on personal circumstances.

Further information and application forms can be obtained by writing (with enclosed CV and brief statement of interests) to the **Director quoting reference no. 89/22: EMBL Grenoble Outstation, c/o ILL, 156X, 38042 Grenoble Cedex, France.** (W6077)A

### INSTITUTE OF BIOTECHNOLOGY, UNIVERSITY OF HELSINKI RESEARCH DIRECTORS

The Institute of Biotechnology, University of Helsinki, is a new inter-faculty research unit with a special Ph.D. training program. At present, about 50 persons are working at the Institute, which has an annual budget of about USD 2.5 million.

The Institute will focus on basic research in different fields of molecular biology essential for the development of modern biotechnology. The research will be carried out in programs led by the Director of the Institute and Research Directors, who have the title of Professor.

The Institute has modern facilities for molecular and cell biology. It has and will have funds available for special equipment needed in new research programs, such as X-ray crystallography.

Applications are invited for research directors in the general field of molecular biology, preferably from those with training in:

- **Plant Molecular Biology**
- **Microbiology**
- **Biological Structures (Crystallography, Electron Microscopy)**
- **Developmental Genetics**

The research directors are appointed for a 5-year period (renewable) but can be made permanent for special reasons. The applicants should hold a Ph.D. or an equivalent degree, a substantial publication record and experience in directing scientific research. The salary is at the level of university professor in Finland (FIM 175,000-225,000; equivalent to USD 41,000-52,000).

Applications should be addressed to the **Board of the Institute of Biotechnology, Registrars Office of the University of Helsinki, Hallituskatu 8, SF-00100 Helsinki, Finland.** The applications should be at the University of Helsinki not later than May 31st, 1989.

Applications should include:

- 1) **curriculum vitae**
- 2) **list of publications 1980-1989**
- 3) **reprints of publications 1985-1989**
- 4) **a 5-year research plan**, with an estimation of the personnel required and possible plans for financing
- 5) **date when the applicant is available**

The Research Directors will be appointed by the Chancellor of the University after consultation with the Scientific Advisory Board of the Institute. The positions will be filled during the autumn of 1989.

For further information please contact Prof. Lauri Saxen (Chairman of the Board; telephone +358 0 4346433) or Prof. Helge Gyllenberg (Acting Director; telephone +358 0 4346041 or telefax +358 0 4346028). (W6031)A



# INTERNATIONAL SYMPOSIUM ON ANTIVIRAL CHEMOTHERAPY

October 1 - 5, 1989 Porto Cervo, Sardinia, Italy

Co-sponsored by  
National Institutes of Health • Consiglio Nazionale delle Ricerche • Università degli Studi di Cagliari  
"Assessorato all'Igiene e Sanità, Regione Autonoma della Sardegna"



## Organizing Committee:

P. La Colla (Chairman) (Cagliari, Sardinia, Italy)  
Y. C. Cheng (Chapel Hill, NC, USA)  
E. De Clercq (Leuven, Belgium)  
G. J. Galasso (Bethesda, MD, USA)  
H. E. Kaufman (New Orleans, LA, USA)  
H. Schellekens (Rijswijk, The Netherlands)  
S. Shigeta (Fukushima, Japan)

## Topics:

Targets for the design of antiviral agents  
Chemistry  
Preclinical pharmacology and evaluation  
Animal models  
Therapeutic trials  
Interferon and combination therapy

## Format:

Lectures • Oral communications • Posters

## Lecturers:

Y. C. Cheng (University of North Carolina, Chapel Hill, NC, USA)  
E. De Clercq (Rega Institute, K. U. Leuven, Belgium)  
F. Dianzani (Università "La Sapienza", Rome, Italy)  
G. J. Galasso (National Institutes of Health, Bethesda, MD, USA)  
R. C. Gallo (National Institutes of Health, Bethesda, MD, USA)  
P. Hardewijn (Rega Institute, K. U. Leuven, Belgium)  
A. Holy (Czechoslovak Academy of Science, Prague, Czechoslovakia)  
H. E. Kaufman (LSU Eye Center, New Orleans, LA, USA)  
E. R. Kern (UAB Medical Center, Birmingham, AL, USA)  
W. H. Prusoff (Yale University, New Haven, CT, USA)  
H. Schellekens (TNO Primate Center, Rijswijk, The Netherlands)  
S. Shigeta (Fukushima Medical College, Fukushima, Japan)

For further information please contact: Prof. P. La Colla - Dipartimento di Biologia Sperimentale, Università degli Studi  
Via Porcell 4, 09124 Cagliari - Italy - Tel. (070)663058 Fax (070)655648

W6095M





# UNIVERSITY OF LEIDEN

Faculty of Mathematics and Sciences, Department of Biology

## professor of ethology (f/m) (full time)

vacancy number: 9-099/2407

The appointee will work within the research group Ethology in the Organismal Zoology. The central research-theme is the causal relations between animal behaviour and both biotic and abiotic environmental factors as well as the role of learning processes in it. Modification of the current research programme is possible in view of the specific expertise and experience of appointee.

### Function and requirements:

- The appointee will organize and develop the teaching and research of Ethology which requires a wide-ranging and up-to-date survey of the field.  
The research-programme will require a quantitative and experimental approach to functional and causal problems;
- in view of the desired collaboration with other research-projects within the department of Biology a strong interest in evolutionary problems is of importance;
- the appointee will likewise become involved in the organization teaching and research.

Further information can be obtained from:  
Prof. dr. J.L. Dubbeldam, tel. 071-275039 and  
from Mrs. dr. L.G. van der Molen, secretary tel.  
071-274982/275174.

Applicants, and also those who wish to suggest potential candidates, are requested to contact the chairman of the Selection Committee:  
Prof. dr. J.L. Dubbeldam, Department of Biology, Kaiserstraat 63,  
P.O. Box 9516, 2300 RA LEIDEN.

Applicants, with the number of the vacancy on the letter and the envelop, should submit within two months a full CV (including the names of three referees?), a list of publications and a description of current research interests to the address above.

(W6073)A

## British-German Academic Research Collaboration Programme ARC

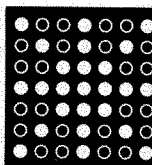
The British Council in Germany invites applications for support under this new programme to promote collaboration between research groups in higher education institutions in Britain and the Federal Republic of Germany.

Priority will be given to research projects in science, engineering and business related studies. Applications in the social sciences will also be considered.

Financial support, contributing towards travel and living costs, will be given either for initial exploratory visits or in support of agreed projects between research groups in the two countries.

ARC is financed jointly by the British Council and the German Academic Exchange Service (DAAD) with additional support from the Anglo-German Foundation for the Study of Industrial Society.

For more information and application forms, British research groups should apply to: The British Council, Science Officer, Hahnenstr. 6, 5000 Cologne 1, West Germany. Tel: 0221-20644-33 Fax: 0221-20644-55. Telecom Gold: 81:BC0024.



The  
British  
Council

(W6069)N

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FOUNDATION FOR RESEARCH AND TECHNOLOGY HELLAS (F.O.R.T.H.)

INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

# POSTDOCTORAL PROGRAMME

The IMBB, founded in 1983, is an independent research and training institution, international in orientation. It is part of the Foundation for Research and Technology — Hellas/ Research Center of Crete, and is funded by the Greek Government, the Mediterranean Integrated Programme of the European Economic Community, and competitive grants. Some information about it can be found in a recent article in *Science* (243: 470-471, 27 Jan. 1989). Its current departments, group leaders and activities are as follows:

**INSECT MOLECULAR GENETICS (F. C. Kafatos, C. Louis, A. Robinson, C. Savakis):** Gene regulation in *Drosophila*; A complete physical map of *Drosophila*; Genetics, molecular biology and engineering of an agricultural pest, the Mediterranean fruitfly.

**MAMMALIAN MOLECULAR GENETICS (N. Anagnou, N. Moschonas, J. Papamatheakis, V. Zannis):** Gene regulation in the MHC locus; human gene regulation and disease (apolipoprotein genes; globin genes/thalassaemias; glutamate dehydrogenase genes/neurodegeneration).

**YEAST MOLECULAR GENETICS (D. Alexandraki, G. Thireos):** Transcriptional and translational control; general control of amino acid metabolism; sequencing and functional analysis of the yeast genome.

**PROTEIN STRUCTURE AND FUNCTION (M. Kokkinidis, K. Petratos, E. Stratakis):** Protein crystallography and modelling; development of structure prediction techniques; enzyme engineering; variants of a biological pesticide (*Bacillus thuringiensis*).

**PLANT AND PLANT PATHOGEN MOLECULAR GENETICS (N. Panopoulos, M. Tabler, M. Tsagris):** Phytopathogenicity genes; plant viruses and viroids; genetic engineering for plant protection and its safety assessment.

**ENZYME TECHNOLOGY (V. Bouriotis):** Methods for protein purification; downstream processing; enzymes for molecular biology.

**IMMUNOLOGY AND DIAGNOSTICS (E. Krambovitis):** Hormone immunodiagnostics; molecular diagnostics and immunodiagnostics for human, animal and plant diseases.

**ELECTRON MICROSCOPY (V. Galanopoulos):** Structure function and differentiation of cytoskeletal elements in developing insect follicles.

The IMBB invites applications for postdoctoral research and training in all of the above groups. Fellowship support is available, but eligible applicants are encouraged to apply for international fellowships (EMBO, EEC, etc), with IMBB support guaranteed as a backup.

Please contact the pertinent group leader(s).

C/o Georgia Houlaki, Administrative Secretary

IMBB/FORTH RESEARCH CENTER OF CRETE

P.O. Box 1527, 711 10 Heraklion, Crete, Greece.

fax: (30) 81 239 735 tel: (30) 81 210 079, (30) 81 210 091

Enclose a curriculum vitae and have three letters of reference sent to the same address.

(W6099)A



# Scientific Computing and Automation (EUROPE)

Conference and Exhibition, 12-15 June 1990, Maastricht, The Netherlands

The 2nd **SCA** conference and exhibition will be held in Maastricht, The Netherlands, an ideal location which takes the meeting into the heart of Europe.

As before, **SCA** is entirely devoted to computing and automation subjects which cross all scientific disciplines; hence the continued theme 'Les Sciences sans Frontières'.

By definition, a conference which must appeal to a multidisciplinary audience will be broad in scope. Under the Chairmanship of Dr. Erkki Karjalainen from Helsinki, **SCA** will bring together specialists from a wide range of disciplines who will devote their attention to contemporary topics likely to be of particular interest to an audience keen to learn from, and exchange views on, a wide range of issues.

**INTERNATIONAL EXHIBITION:** An international exhibition will complement the SCA conference - both of which will be held under one roof. The exhibition will be organised by the publishers of *Nature* magazine.

The topics for **SCA (EUROPE) 1990** will include:

- Chemical applications for supercomputers
- Sampling strategies and experimental design
- Online databases in chemistry
- Databases for spectroscopy
- Dynamic models for catalysis, processes and metabolism
- Expert systems and statistical tools for the interpretation of laboratory data
- Robotic and discrete automation in the laboratory
- Interfacing tools; software and hardware tools
- Chemical monitoring - multivariate sensors and biosensors
- LIMS and LAN strategies for the laboratory
- Workstations for scientists - RISC, parallel and traditional
- Scientific applications for neural networks and fractals
- Software toolkits for exploratory data analysis and mathematics
- Computer graphics and image analysis
- Designing molecules by computer

For further information, contact:

**SCA (Europe), Reunion International**  
W.G. Plein 475

1054 SH Amsterdam, The Netherlands

(W6108)C



## GLAXO INSTITUTE FOR MOLECULAR BIOLOGY S.A.

The Geneva molecular biology research unit of one of the largest pharmaceutical companies in the world has a vacancy for

### POSTDOCTORAL FELLOWSHIPS

The Glaxo Institute for Molecular Biology (GIMB) has a staff of approximately 130. Projects include viral therapeutics, neurobiochemistry and the regulation of cell proliferation and differentiation. We are part of the Glaxo group, Britain's largest pharmaceutical organization, which has an outstanding reputation for the quality of its research and products. GIMB is a leading group in applying genetic engineering to medical research.

Applications are invited for Research Fellowships in the following project areas:

- regulation of IgE synthesis
- molecular neurobiology
- cytokine signal transduction
- fungal molecular biology

Candidates should have appropriate experience in molecular or cell biology.

We offer excellent facilities and research opportunities together with an attractive salary and benefits package. Interdisciplinary and collaborative research is actively encouraged at GIMB.

If you are interested in this position, please send your curriculum vitae with a list of publications and the names of three references to: Rita Gloor, Personnel Manager, GLAXO IMB S.A., 46, route des Acacias, 1211 Geneva 24, Switzerland.

(W6078)E



## NEW DATES

## NEW VENUE

# INTERNATIONAL TNO MEETING ON ANIMAL MODELS IN AIDS

Maastricht, the Netherlands

23-26 October 1989

The emphasis of the Meeting will be on the validation of models for human HIV infections, comparison of the different models, pathology and immunology, pathogenesis, antiviral agents and vaccine development. In conjunction with the Meeting an exhibition will be organised.

### Organizing Committee:

M. Horzinek, chairman

G. Galasso, J. Goudsmit, G. Hunsmann, H. Lutz, W.J.C. Melgert, L. Montagnier, N. Pedersen, H. Schellekens

The sessions will be devoted to: HIV-1 and HIV-2 in animal models; Mouse models; Simian retroviruses; Feline retroviruses; Large animal models. The Meeting will start with a general session on AIDS and will be concluded by a round up session. Each session will be introduced by one or more introductory lectures. There is ample opportunity to present free papers and posters. The introductory lectures include E. de Clercq, R. Desrosiers, J. Eichberg, M. Gardner, M. Gonda, J. Goudsmit, O. Jarret, P. Marx, J. McGowan, L. Montagnier, R. Montelaro, O. Narayan, N. Pedersen.

For information and to obtain abstract forms please contact:

**Ms. S. van de Graaf**

**TNO Corporate Communication Department**

**P.O. Box 297**

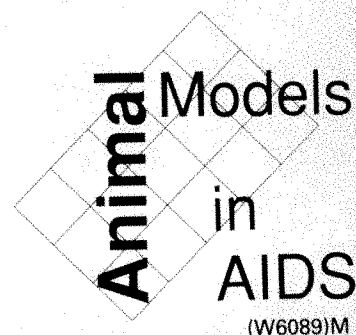
**2501 BD The Hague**

**The Netherlands**

**Phone: +31-70496612/11**

**Telex: 31660 tnogv nl**

**Telefax: +31-70821433**



## BECKMAN SYMPOSIUM ON ONCOGENES

PARIS, MAY 11-12th 1989

Inter-Continental Hotel 3, rue de Castiglione Paris 1



### SCIENTIFIC ORGANIZERS

D. STEHELIN, Lille France and A. LEVINE, Princeton USA

### SESSION 1: GENE REGULATION

J. Ihle, Memphis • R. Tjian, Berkeley • I. Verma, San Diego  
P. Vogt, Los Angeles • B. Wasylyk, Strasbourg • M. Yaniv, Paris

### SESSION 2: ANTI-ONCOGENES

S. Benchimol, Toronto • E. Harlow, Cold Spring Harbor • P. Howley, Bethesda  
A. Levine, Princeton • D. Livingston, Harvard • A. Tavitian, Paris

### SESSION 3: RECEPTORS

P. Charnay, Paris • R. Evans, San Diego • C.H. Heldin, Uppsala  
J. Pouyssegur, Nice • C. Sherr, Memphis • P. Tiollais, Paris

### SESSION 4: COOPERATION OF ONCOGENES

F. Alt, New York • M. Barbacid, Princeton • F. Cuzin, Nice  
D. Lowy, Bethesda • E. Stavnezer, Cincinnati • D. Stehelin, Lille

INFORMATION: Sylvie Foncke  
BECKMAN FRANCE  
Tel: (1) 43 81 93 00  
Telex 230 191 F  
Fax (1) 43 81 16 80

**BECKMAN**

INSTITUT PASTEUR, LILLE

CNRS

INSERM

(W6102)M

## THE EUROPEAN SCHOOL OF HAEMATOLOGY

### PROGRAMME 1989

22nd-26th May

ALLOGENEIC BONE MARROW TRANSPLANTATION  
E. GLUCKMAN, T. GORDON SMITH, B. SPECK

28th-29th June

ACUTE AND LATE EFFECTS OF IRRADIATION ON THE  
HAEMOPOIETIC SYSTEM

Satellite Symposium of the 17th International  
Congress of Radiology

M. TUBIANA, C. PARMENTIER

2nd-6th October

FIBRINOLYSIS

F. BACHMANN, M. SAMAMA

6th-10th November

DREPANOCYTOSE ET  $\beta$  THALASSEMIE

Session Spéciale en Français

Y. BEUZARD

20th-23rd November

The first Applied Technology Session: (in French)  
BIOLOGIE MOLECULAIRE:

Méthodologies; Applications à l'Hématologie  
M. GOOSSENS

REGISTRATION AND INFORMATION:

E.S.H.

Hôpital Saint Louis — Centre HAYEM

1, avenue Claude Vollefaux

75475 PARIS Cedex 10

FRANCE

Téléphone: (1) 4206 32 06 — Telex: IRM 212 309

Telefax 42-41-14-70

(W6103)M

# RHONE-POULENC SANTE

The health division of France's principal chemical group

Invites applications from experienced molecular biologists for three RESEARCH SCIENTIST posts in its newly built Biotechnology Institute near Paris.

## Streptomyces molecular genetics

This position requires a Ph.D. in bacterial molecular biology as well as a minimum of 3-4 years' post-doctoral experience in the Streptomyces field. Familiarity with recombinant DNA technology in Streptomyces microorganisms, as well as experience in cloning genes involved in secondary metabolism, is essential. Réf. 50425/A.

## Mammalian cloning technology

Qualified candidates for this position will have a Ph.D. and good post-doctoral experience in the cloning and analysis of mammalian cDNAs or genes. A proven record of achievement using up-to-date DNA cloning strategies (construction of cDNA libraries in various vectors: screening by oligonucleotide hybridization, immunodetection or expression, subtractive hybridization...) is essential. Réf. 50425/B.

## Transgenic animals

This position requires a Ph.D. level in cellular and molecular biology and a good post-doctoral experience in the production and use of transgenic animals. The candidate should be able to develop a small unit for the production and genetic analysis of transgenic animals. Réf. 50425/C.

Please send your full resume quoting the reference to Media-System, 6/8 impasse des Deux Cousins, 75849 Paris Cedex 17, France.

(W6053)A



## Universität Witten/Herdecke

Postdoctoral Position  
in Biochemistry/  
Molecular Biology

to join project on site-directed mutagenesis of proteins and participate in teaching. Experience in recombinant DNA techniques essential. Send application (with 2 references) before May 20 to:

Prof. Dr. W. Wintermeyer  
Universität Witten/Herdecke  
Stockumer Straße 10  
D-5810 Witten. (W6081)A

# nature

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## FIRST ANNOUNCEMENT

September 20 . 21 . 22, 1990  
BORDEAUX, FRANCE

### First International Congress on Very Ultra Low dosage

Scientific program: Plenary lectures,  
Oral Communications sessions:

- |                             |                      |
|-----------------------------|----------------------|
| ☆ Biochemistry              | ☆ Molecular biology  |
| ☆ Biophysics                | ☆ Physical-chemistry |
| ☆ Clinical pharmacology     | ☆ Physiology         |
| ☆ Experimental pharmacology | ☆ Toxicology         |
| ☆ Hematology                | ☆ Virology           |
| ☆ Immunology                |                      |

Deadline for abstracts: March 1, 1990.

Further inquiries regarding Scientific Abstracts should be directed to:  
Professor Ch. DOUTREMEPUICH, Hematology Laboratory,  
Faculty of Pharmacy, 3, Place de la Victoire, 33076 BORDEAUX,  
France.

Correspondence to the Congress: VULD Congress, Secretariat,  
Hematology Laboratory, Faculty of Pharmacy, 3, Place de la  
Victoire, 33076 BORDEAUX, FRANCE.

Tél: 33 56 91 34 24 EXT 711

Fax: 33 56 94 31 28

(W6063)Q

# EMBL

The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, West Germany and outstations in Hamburg and Grenoble, has the following vacancy:

## RESEARCH ASSISTANT

The successful candidate will work with the Scanning Transmission Electron Microscope (STEM). This project will involve a combined structural and biochemical study of mammalian splicing complexes and SnRNP particles.

We offer an above-average salary, plus certain allowances depending on personal circumstances.

Please write briefly for an application form, quoting reference no. 89/20, to:

EMBL, Personnel Section, Postfach 10.2209, D-6900 Heidelberg,  
Federal Republic of Germany. (W6067)A

# TEMA 7

The 7th International Symposium on Trace Elements in Man and Animals will be held in Dubrovnik, Yugoslavia

20-25 May 1990

Details available from the following sources:—

TEMA 7 Local Committee  
c/o Institute for Medical  
Research & Occupational Health  
M. Pijade 158  
41000 Zagreb  
Yugoslavia

TEMA International Secretariat  
Rowett Research Institute  
Greenburn Road  
Bucksburn  
Aberdeen AB2 9SB  
Scotland

TEMA INTERNATIONAL (9128)M

Sandoz Ltd., Basle, Switzerland, one of the world's leading pharmaceutical companies, is offering a one-year

# Postdoctoral Fellowship

in glycoprotein research and development.

The successful candidate will work in a highly motivated research and development environment. Main task of the candidate will be to establish efficient polysaccharide characterisation methods and procedures and carry out research on biotechnically produced glycoproteins.

Applicants require a Ph.D. or an equivalent in Biochemistry and/or related sciences. Remuneration will be based on common standards.

Interested candidates with experience in carbohydrate chemistry should submit their curriculum vitae, names of references and a list of publications as soon as possible, but at latest by June 15th to:

**Sandoz Ltd.,  
attention Mr. R. Zbinden, Personnel Department,  
Ref. 9803  
CH-4002 Basel, Switzerland**



(W6075)A

## EUROPEAN TECHNOLOGY INFORMATION RESEARCH

Asahi Chemical Industry Co. Ltd. is a major Japanese chemical company. It's products cover synthetic fibres, industrial chemicals and petrochemicals, plastics and rubber, food products, pharmaceuticals and medical equipment, electronics, construction materials and housing.

Research and Development, Europe, is responsible for supporting Asahi Research and Development in Japan by providing European new technology and market information.

The Director of R & D, Europe now requires a young science graduate (early to mid-20's) who is interested in all aspects of science, to work as his Assistant in our small, friendly, central-London offices.

The post involves monitoring new European technology, that would be of interest to Asahi, in various fields including biotechnology, electronics and new materials.

The work includes information research, attendance at meetings and conferences and preparation and submission of reports and new technology proposals. Support for current company research would also be required.

Candidates should have a degree in a scientific discipline with 2-3 years experience of information work. Candidates would also be expected to have a high degree of initiative and the ability to work alone as the European Director is frequently away on business.

Computer literacy, together with knowledge of one or more European languages, would be an advantage. Interest in Japan and Japanese business would also be useful.

Salary is commensurate with age and experience. Financial support in learning Japanese would also be considered.

If you are interested in the above position, please send a full CV stating current salary to:

**Mr S. Miyata**

**European Director, Research and Development  
Asahi Chemical Industry Co. Ltd.**

**54 Grosvenor St., London W1X 9FH. (W6080)A**



**BIOPROBE  
S Y S T E M S**

## INTERNATIONAL WORKSHOP ON MEDICAL MOLECULAR BIOLOGY

**July 3-8, 1989**

**Sept 4-9, 1989**

**PARIS-FRANCE**

An intensive lecture and "hands-on" laboratory course covering the following topics:

- the non-radioactive Nucleic Probes Technology
- *In Situ* Hybridization (Papillomavirus)
- Gene amplification by the Polymerase Chain Reaction

Number of participants limited to 12

For further information and registration details please contact:

**BIOPROBE SYSTEMS, 26 bis rue Kléber,  
93100 MONTREUIL-FRANCE**

**Phone 33.1.48.51.66.22**

**Fax 33.1.48.51.59.90**

**BIOPROBE SYSTEMS, Molecular Biology applied to  
Research and Diagnostics (W6101)V**



**Sandoz is a leading pharmaceutical company with an active research commitment in the field of cardiovascular medicine. We are looking for a**

# CELL BIOLOGIST

to join a new group within our cardiovascular department. As part of an interactive team the work will involve a basic research effort using cellular and molecular biology to address the pathology and treatment of cardiovascular disease.

The successful candidate will be in charge of his/her own laboratory and will be responsible for the establishment, characterization and maintenance of primary cultures and cell lines. Experience with endothelial cells/hepatocytes, immunohistochemical and in situ hybridization techniques would be an advantage.

Candidates who have a PhD or MD with research or industrial experience are invited to send a curriculum vitae including a list of publications, references and a statement of research interests to:

**Sandoz Ltd., Personnel Department, Ref. 9103,  
attention Dr. R. Racine (061 24 24 66)  
CH-4002 Basle, Switzerland**



(W6074)A

## University of Utrecht The Netherlands

Faculty of Biology

vac.nr.  
146.183

### professor of comparative endocrinology

Applications are invited for the chair of Comparative Endocrinology in the Department of Experimental Zoology. The research group presently performs research on the endocrine regulation – both physiologically and cell biologically – of the reproduction in teleosts. The group collaborates with other research groups of the Department of Experimental Zoology, with the Department of Fish Culture and Fisheries of the Agricultural University in Wageningen and with well-known scientists abroad.

Applicants need to have a broad knowledge and experience in comparative endocrinology and to be acquainted with modern physiological and cell biological methods.

The candidate should be willing to teach at pregraduate and postgraduate level. He or she should be willing to participate in administrative functions at different levels of the University organization.

The salary will be maximum Dfl. 9957,— per month.

Applications, including curriculum vitae, list of publications and the names and addresses of two referees, should be sent within 3 weeks to the Dean of the Faculty of Biology, prof. Dr. G.A. van Arkel, Office of the Faculty of Biology, Padualaan 14, 3584 CH Utrecht, The Netherlands: Phone 030-533133/532276.

Equal employment, irrespective of sex, is university policy.

Those wishing to recommend suitable candidates are requested to inform the Dean of the Faculty of Biology.

(W6068)A

### Hildegard Doerenkamp and Gerhard Zbinden Foundation for Realistic Animal Protection in Scientific Research Scientific AWARDS 1989 and 1990

A prize of DM 50.000,— each will be awarded for outstanding scientific contributions to the following topics:

#### Award 1989:

#### "Alternatives for Animal Experimentation in the Neurosciences and in Epilepsy Research"

Applications should consist of published or unpublished reports on alternative methods replacing animal experimentation in the neurosciences and in epilepsy. New techniques that can reduce the number of higher animals used (primates, dogs, cats) or decrease experimental stress in these animals will also be considered.

#### Award 1990:

#### "Anesthesia in Laboratory Animals— Management of Chronic Pain in Laboratory Animals"

Applications should consist of publications, manuscripts or audio-visual presentations, detailing concepts, application and success of new or refined methods leading to the reduction of pain and suffering of experimental animals in acute and chronic experiments.

Applications should be sent to:

#### Award 1989:

Prof. Dr. Diether Neubert  
Institut für Toxikologie  
und Embryopharmakologie  
Freie Universität Berlin  
Garystrasse 5  
D-1000 Berlin 33

**Deadline: February 28, 1990**

#### Award 1990:

Prof. Dr. Kay Brune  
Institut für Pharmakologie  
der Universität  
Erlangen-Nürnberg  
Universitätsstr. 22  
D-8520 Erlangen

**Deadline: February 28, 1991**

All materials remain the property of the applicants and will be returned within three months after distribution of the prize. The jury reserves the right to split the prize among not more than three applicants. No special application forms are required.

(W6083)N

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congratulate

*James E. Birren*  
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*J.C. Brocklehurst*  
*M.D., Great Britain*

*David Danon*  
*M.D., Israel*

*G.S. Roth* / *J.A. Joseph*  
*Ph.D., U.S.A.* / *Ph.D., U.S.A.*

on their being awarded the

1989 SANDOZ PRIZE  
FOR GERONTOLOGICAL RESEARCH

by the Jury of the International Association of Gerontology

With this prize, the IAG honors distinguished personalities  
for their achievements in gerontological research and  
leadership in activities for the betterment of life of elderly  
fellow citizens in all parts of the world.

# Sandoz Prize for Immunology

The prize will be worth US \$ 100 000 (US \$ 20 000 personal recognition/US \$ 80 000 support for research programme), and will be sponsored by SANDOZ LTD., Basle, Switzerland with the purpose of encouraging research in all areas of immunology with special emphasis on clinical immunology, including autoimmune diseases, cancer immunology, immunity to infectious diseases, transplantation immunology and discoveries in immunology leading to therapeutical applications.

Members of the Jury are G. Ada, J.-F. Bach, T. Honjo, P. Marrak, H.O. McDevitt, J.J. van Rood, R. Zinkernagel, and two representatives of Sandoz Ltd. The prize will be awarded on the occasion of an important Immunology Meeting in 1990.

Applications in English should comprise a summary of the research work of 3–5 pages, curriculum vitae, bibliography, experimental original papers separate from reviews and chapters, and reprints of not more than 3 key published papers in English or with extended summaries in English.

Individuals and research teams are invited to submit their applications not later than 30th June, 1989 to Sandoz Prize for Immunology, P.O. Box 182, 4013 Basel, Switzerland.



(W5985)N

## Karolinska Institute

Department of Molecular Biology

### POSTDOCTORAL POSITION

#### Molecular Biology of Membrane Protein Assembly

We have an immediate opening for a postdoc interested in working on problems related to protein sorting and membrane protein assembly (see PNAS 85, 3363-3366 and BBA 947, 307-333). Our department is wholly focussed on this field, using current methods of molecular and cell biology such as site-specific mutagenesis and *in vitro* transcription-translation assays. Applicants should therefore have a good background in molecular-biology or microbiology.

Salary is 5500 Skr per month tax free (approx £500); in addition, apartment rent, health insurance, and travel costs are covered. The Department of Molecular Biology is a part of the Karolinska Institute and is located alongside the Karolinska Center for Biotechnology in Huddinge, south of Stockholm.

Please address all enquiries to: **Dr. Gunnar von Heijne, Department of Molecular Biology, Karolinska Institutet, Huddinge Hospital-K87, S-141 86 Huddinge, Sweden. Phone: Int + 46-8-774 7699 (W6091)A**

The International Association of Biological Standardization will hold its 21st Congress at

Annecy, France on October 4-6, 1989, with the title:

### "PROGRESS IN ANIMAL RETROVIRUSES."

The subject will be exclusively devoted to the:

1. retrovirus status of cells and animals used for assays,
2. evaluation of detection methods,
3. immunization and biotherapy approaches, and
4. relevance to human retroviruses infections.

For further information, you may contact:

**Dr. Daniel Gaudry**  
Secretary of the 21st Congress of the IABS  
254, rue M. Merieux  
69007 LYON – FRANCE  
Tel: 33.72.72.30.25  
Fax: 33.72.72.33.68

(W6070)A

## RESEARCH GROUP LEADER IN MOLECULAR BIOLOGY/VIROLOGY

Our team is primarily engaged in research on experimental antiviral chemotherapy, concentrating on evaluation of efficacy and mode of action and cellular kinetics of new antiretroviral drugs. The laboratory supports ongoing and future clinical drug evaluation trials (HIV cultures, development of PCR) and collaborates with other research groups at the University of Zürich and the Federal Institute of Technology (ETH).

The ideal candidate (Ph.D. required) is expected to act as group leader and should have demonstrated experience in the performance and management of basic research projects. Knowledge of the German language is not an essential, but helpful prerequisite.

The position is available from July of 1989. Please send curriculum vitae with names and addresses of three references to: **Ruedi Lüthy, M.D. Chief, Division of Infectious Diseases, Department of Medicine, University Hospital, CH-8091 Zurich, Switzerland. (W6097)A**

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## Protein Purification Courses

### Practical Course Programme 1989

#### – European Consultancy –

**1. Strategies of Recombinant Protein Purification**  
recombinant human Superoxide Dismutase (rh SOD)

4-day practical course including process development optimization, scale up, and analytical purity checking techniques

**Dates:** May 29-June 2, in English  
December 4-8, in English  
December 11-15, in German

**2. Downstream Purification: Aspects of Validation of Chromatographic Processes**

One and a half day theoretical seminar on validation strategies of process chromatography. Aimed at people responsible for process design, pilot plant, and production facilities

**Date:** open

**For further information please contact:**

**Pharmacia LKB Biotechnology**  
**European Consultancy, Birgit Roth**  
**Munzinger Strasse 9, D-7800 Freiburg**  
**West Germany**  
**Telephone: 49-761-4903152**  
**Telefax: 49-761-4903159**

**3. Protein Purification, Process Development, Optimization, and Scaling up**

4-day practical course leading to production scale chromatography (42 litre columns)

**Dates:** July 17-21, in Italian  
September 18-22, in French  
October 23-27, in English  
October 30-November 3, in English  
November 6-10, in German

**4. Process Scale Practical Course on Monoclonal Antibody Purification**

2-day course using new dedicated monoclonal purification media, software package, and instrumentation

**Dates:** June 15-16, in English  
October 16-17, and 19-20, in English  
November 27-28, 1989, in German  
November 30-December 1, in English



**Pharmacia**

(W6079)C

### INTERNATIONAL CONFERENCE

#### "THE GASTROINTESTINAL EPITHELIUM"

**September 24-28, 1989, RIOM, France**

**Organizing Committee:** K. Haffen, M. P. Hauri, M. Laburthe, M. J. M. Lewin, D. Swallow, G. Willems.

**Program:** Cell biology and molecular genetics of differentiation. Differentiation markers. New models for the study of GI epithelial cell differentiation. Maturation, growth and cell renewal. Cell renewal and carcinogenesis. Mechanisms and regulations of transport. Receptors. Interactions between the gastrointestinal epithelial cells and the immune system.

Further details: Nature, 1989, April 6, classified 29.

**Information:** M. J. M. Lewin, "The GI Epithelium", INSERM U.10, Hôp. Bichat, 75877 PARIS CEDEX 18, France. Tel. (1) 40.25.83.90 & (1) 40.25.83.93. Fax (1) 46.27.85.36. (W6065)C

### AUTOMATED DNA SEQUENCING

**Oslo, September 18 - 23, 1989**

A practical course where three different fluorescence-based DNA sequencers as well as a film reader will be available for the comparative analysis of samples brought by the participants. Teachers include Wilhelm Ansorge, Alan Bankier, Lennart Philipson, Peter Rice, Cassandra Smith, Brian Sproat and Tom Kristensen.

The course will this give a good theoretical background for modern sequencing methods as well as hands-on experience with sequencers currently in or near the market.

Applications before June 1 to: Tom Kristensen, Research Institute for Internal Medicine, Rikshospitalet, 0027 OSLO 1, Norway. Telefax: (02) 201401. (W6076)C

### THE PARENTERAL DRUG ASSOCIATION PRESENTS COURSES IN FRANKFURT FOR THE HEALTH CARE INDUSTRIES FROM JUNE 12-JUNE 16 1989

1. From gene to product: fundamentals of biotechnology
2. Biotechnology plant design
3. Aseptic processing audits
4. Sterile manufacture with blow/fill/seal technology
5. Clean room management
6. Fundamentals and concepts of calibration and metrology
7. Validation of aseptic pharmaceutical processes
8. Validation of non-aseptic pharmaceutical processes

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**Kennet Bioservices (UK)**  
**Fax 0793617415**  
**Telex 444502 or**  
**Telephone 0793 826267**

(9088)C

# RESEARCH COORDINATORS

## THE COMPANY

Innogenetics is a leading European biotechnology company employing over 100 highly qualified researchers. In a creative research environment, they are applying recombinant DNA technology to develop innovative drugs and diagnostics. Important areas of research are Alzheimer disease, Aids, Atherosclerosis, Wound Healing and Bone Disorders.

## THE JOB

Innogenetics has now openings for several research coordinators in the field of infectious diseases, cardiovascular diseases, neurological diseases, clinical immunology and endocrinology. Reporting to the scientific director, the successful applicants will co-ordinate the activities of the various on-site research efforts with those of collaborative projects in external laboratories. The research coordinators will optimise the use of human and financial resources and participate in the selection of new projects in their particular field of research. They will also establish and manage the necessary academic interactions to explore the potentials of new in-house developed molecules and interact with Innogenetics' business department.

## THE CANDIDATE

Successful applicants will have acquired a Ph.D. or M.D. qualification and have at least 5-10 years postdoctoral experience in one of the above mentioned research areas. Previous management of a scientific team or leadership of an academic research laboratory is highly desirable. Exceptional communication, interpersonal and supervisory abilities are also necessary as well as a thorough understanding of modern biotechnology and working knowledge of patent regulations. Fluency in English is a prerequisite and a working knowledge of Dutch, French and German would be helpful.

## THE OFFER

Innogenetics offers a remuneration in keeping with the importance of this position, including a benefits package and relocation assistance in addition to an excellent salary. The extremely pleasant research headquarters of Innogenetics is located in Ghent, an attractive middle-age city in the heart of Europe and only 40 miles away from the Belgian coast and neighboring countries.

Qualified applicants should send a c.v., including references, to **Personnel Division, Innogenetics N.V., Industriepark Zwijnaarde 7, bus 4, 9710 GENT, Belgium.** (W6100)A

### University of Geneva announces an opening for a position of a FULL PROFESSOR in

#### COMPUTATIONAL CONDENSED MATTER PHYSICS

The new professor will have the responsibility for research and teaching in computational condensed matter physics. He will in particular have the task to develop the research activities of the "Institut Romand de Recherches Numériques en physique des Matériaux" (IRRMA) situated at the campus of the "Ecole Polytechnique Fédérale de Lausanne". He will assume the direction of this Institute for a certain number of years and it is also expected that he actively participates in the scientific and academic life of the University of Geneva.

The applicant should have a Ph.D. in physics or an equivalent degree. Experience in teaching, leading and management of research groups as well as some experience in university administration is desirable. Letters of application, a curriculum vitae and a list of publications should be addressed before June 30 1989 to:

**Secretariat de la Faculté des Sciences,  
20, quai Ernest-Ansermet,  
CH-1211 Geneva 4, Switzerland**

where additional information may be obtained.

(W6072)A



## EUROPEAN SOUTHERN OBSERVATORY

Organisation Européenne pour des Recherches Astronomiques dans l'Hémisphère Austral Europäische Organisation für astronomische Forschung in der südlichen Hemisphäre

## FELLOWSHIP AT LA SILLA

A position is available at La Silla for a post-doctoral fellow with an interest in observational astronomy. Experience with IR instrumentation or optical photometry will be an advantage.

The successful applicant will be expected to spend not more than 50% of his/her time in support related activities and the rest of the time doing scientific research. The presence at La Silla will be for at least 150 nights per year. ESO fellowships are granted for a period of one year normally renewed for a second period and exceptionally renewed for a third and final year.

The facilities on La Silla consist of 15 telescopes including the SEST 15-m submillimeter antenna, and the new 3.5m NTT. The computing facilities comprise an HP1000 system with full image processing capabilities (IHAP), a VAX 11/750 mainframe, and three SUN 4/110 workstations for image processing (MIDAS).

Close to 20 astronomers, including staff members, fellows and students, work at La Silla. The research projects currently pursued by the astronomical staff at La Silla include low mass star formation (Herbig-Haro objects, molecular outflows, T Tauri stars), OH/IR stars, symbiotic stars and proto-planetary nebulae, coronal activity in late type stars, chemistry of molecular clouds, formation of massive stars, and starburst activity, dynamics of elliptical galaxies, active nuclei, QSOs and gravitational lensing, and observational cosmology.

Applicants normally should have a doctorate awarded in recent years. The monthly basic salary will be not less than DM 4190 to which are added 7% for pension purposes and a non-resident allowance of 30-45% as well as a mountain allowance of 5-10%. Applications should be submitted to ESO not later than May 15, 1989. Applicants will be notified in July 1989. The ESO Fellowship Application Form should be used and be accompanied by a list of publications. In addition, three letters of recommendation should be obtained from persons familiar with the scientific work of the applicant. These letters should reach ESO not later than May 15, 1989.

Enquiries, requests for application forms and applications should be addressed to: **European Southern Observatory, Fellowship Programme, Karl-Schwarzschild-Straße 2, D-8046 GARCHING b. München, Federal Republic of Germany** (W6094)E

## UNIVERSITE DE LAUSANNE

Professor of Anatomy  
Faculty of Medicine

A full-time is opened for an established scientist capable of teaching macroscopic and topographical anatomy to medical students. Teaching at undergraduate levels is in French but a period of adjustment can be granted.

The successful candidate is expected to develop a strong, independent and externally fundable research program. Collaborative opportunities exist in several areas including neurobiology.

The letter of application, curriculum vitae, description of current and future research plans of most significant publications should be sent copies by July 1st, 1989 to: **M. le Professeur Y. Saudan, Doyen de la Faculté de Médecine, rue du Bugnon 9 - CH - 1005 Lausanne.** (W6109)A

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# Our reputation in your hands

## Senior Quality Assurance Assessors

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Already one of Britain's leading pharmaceutical companies, the Beecham Group is expanding its research and development programme. This has created vacancies for Senior Quality Assurance Assessors whose key responsibility is to ensure that our work achieves the demanding standards dictated by the regulatory authorities, and that new compounds have the highest possible chance of success. These roles will have important implications for Beecham's future.

You will audit data, carry out study inspections, assess facilities and interpret national and international guidelines to meet GCP standards. Your background will ideally be in QA auditing to GLP/GCP standards, clinical research or nursing. Strong personal and analytical skills will help you influence international clinical teams – skills that should

include the confidence to operate independently when the occasion demands it. A driving licence is necessary as the work will involve travel, including overseas site visits as required.

In return we will provide an excellent starting salary, a non-contributory pension scheme, free life assurance and, after a qualifying period, an attractive Company bonus. Generous relocation assistance may be available in appropriate circumstances.

Please send a full CV, including current salary and quoting reference 13/89, to the Personnel Manager, Beecham Pharmaceuticals Research Division, Honeypt Lane, Stock, Essex CM4 9PE, or ring (0279) 622624 (24 hour answering phone) for an application form.

# Beecham

## Pharmaceuticals



(9134)A

### Faculty Position Department of Pathology McMaster University

The Department of Pathology at McMaster University seeks a research scientist to fill a faculty position in the newly established Hemoglobino-pathy Molecular Diagnostic Laboratory. The candidate should have a Ph.D. degree and demonstrated ability to carry out independent as well as collaborative research in molecular genetics and/or developmental biology, broadly related hemopoietic disorders.

The appointment will carry an appropriate faculty rank in the University at the level of Assistant/Associate Professor, will be for an initial period of three years, and carry a salary commensurate with prior experience and qualifications.

Please forward an application with curriculum vitae, names of two referees, and summaries of current and future research plans to **Dr. David H.K. Chui, Department of Pathology, McMaster University Health Sciences Centre, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. (NW3659)A**

### MEDICAL RESEARCH COUNCIL TUBERCULOSIS AND RELATED INFECTIONS UNIT POSTDOCTORAL IMMUNOLOGIST

Applications are invited for the appointment of a postdoctoral (non-clinical or clinical) MRC staff scientist for a period of 3 years with the possibility of an extension of up to 5 years. The candidate is expected to have experience in basic immunology and an interest in the immunobiology of infections with intracellular bacteria. The proposed research project is to study the specificity and MHC-restrictions of the human immune repertoire to mycobacterial protein antigens. Antigens and epitopes relevant to protection or pathogenicity are to be identified by the assay of CD4 T cells in infected healthy and diseased individuals to antigenic fractions and synthetic peptides. Technical and collaborative support for the project will be available within and outside the Unit. Applications and inquiries should be addressed to **Dr. J. Ivanyi, Director of the MRC Unit, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 0HS, Telephone: 01-740-3161, FAX, 01-743-3987. (9098)A**

### POST-DOCTORAL RESEARCH

#### in Protein Biochemistry/Molecular Palaeontology

Applications from the UK and overseas are invited for a three-year post-doctoral research position from late 1989 to investigate proteins from shells and bones and their preserved remnants in fossils. The work will be carried out in new purpose-built laboratories housing a complete range of biochemical equipment, including amino acid analyser and micro-sequencer (ABI), liquid chromatography (Waters 650), and facilities for polyclonal and monoclonal antibody production (Dynatech). The successful applicant will join a rapidly-expanding research group which has already demonstrated the considerable evolutionary and palaeontological significance of skeletal molecules in one invertebrate phylum and has now received funding to allow detailed biochemical investigation in a wider range of taxa. Expertise in purifying and determining the structure of proteins is essential, and the Post-Doc will also be expected to assume some responsibility for the day-to-day running of the laboratories, and to help train new personnel. A competitive salary will be offered, and applicants should send a complete cv including the names of two academic referees to **Dr G.B. Curry, Dept of Geology and Applied Geology, University of Glasgow, Glasgow, G12 8QQ, Scotland (Tel: (041) 330 5444; Fax: (041) 330 4808; E-mail: gcurry@uk.ac.glasgow.geology). (9089)A**

### ASSISTANT PROFESSOR

#### Department of Ophthalmology and Visual Science Yale University School of Medicine

Full time academic appointments are available for independent investigators with the possibility of joint appointments with basic science department. Areas of interest include, but are not restricted to, photoreceptor-RPE interactions, immune functions of ocular cells, axonal transport and regulation of RPE cell proliferation and motility. Excellent facilities and environment are available to allow development of a vigorous research program using modern cellular and molecular approaches. Please send curriculum vitae, statement of research interests, and names of three references to: **Chairman, Search Committee, Department of Ophthalmology and Visual Science, Yale University School of Medicine, P.O. Box 3333, New Haven, CT 06510-8061. Affirmative Action/Equal Opportunity Employer. (NW3652)A**



**UNIVERSITY OF SOUTHAMPTON**  
Department of Biochemistry  
**POSTDOCTORAL RESEARCH FELLOW**

Applications are invited for a Postdoctoral Fellow to join a group studying the insulin receptor tyrosine kinase and the role of protein phosphorylation in cellular transduction of the insulin signal. The focus of the project will be the identification, structure and regulation of protein (phosphotyrosyl) phosphatases active in this system. Experience in one or more of the following: protein purification, enzymology or cell biology, would be an advantage.

The appointment is for a period of three years and will be at an appropriate point on the University Grade 1A scale. The post is funded by the British Diabetic Association.

Applications, including a curriculum vitae and the names and addresses of two referees should be sent to **Dr G. J. Sale, Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton SO9 3TU.** (9125)A

**POSTDOCTORAL FELLOW NEUROSCIENCES**

Intracellular electrophysiological in vitro experience preferred. Join large multi-disciplinary research group investigating pathophysiology of traumatic brain injury. Research to include studies of hippocampal function after injury. Opportunities to develop innovative research program with close linkage to molecular studies. Minimal appointment of 2 years. Salary negotiable. Contact:

**Stephen J. Goldberg, Ph.D., Department of Anatomy, Box 709, Medical College of Virginia/ Virginia Commonwealth University, Richmond, VA 23298. Telephone: 804-786-9529.**

(NW3648)A

**THE UNIVERSITY OF LEEDS DEPARTMENT OF PHYSICS LECTURER (N.A.A.S. funded)**

Applications are invited for the above post available from 1 September 1989. The lecturer will be expected to carry out research in the field of experimental ultra high energy gamma ray astronomy based at Haverah Park.

Salary will be on the Lecturer Grade A scale (£9260-£14500) or Grade B (£15105-£19310), according to qualifications and relevant experience.

Application forms and further particulars may be obtained from and completed applications forwarded to the Registrar, The University, Leeds LS2 9JT, (tel (0532) 333963) quoting reference no. 52/48. Closing date for applications 18 May 1989. (9108)A

**UNIVERSITY OF MANCHESTER DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY**

**POSTDOCTORAL RESEARCH ASSOCIATE**

Applications are invited for a 3-year post based in the School of Biological Sciences and financed by the Wellcome Trust to study the biochemistry of the extracellular matrix in normal and diseased connective tissues. Work will involve studying the interaction of connective tissue components with fibrils formed *in vitro* from normal and mutated collagens using a newly developed system. Experience in protein purification and physical biochemical methods would be an advantage but not essential. Starting date September 1st. Salary range £9,865-£11,680 p.a. Superannuation. Applications (in duplicate) with a full c.v. and the names of two referees to: **Dr K. E. Kadler, Department of Biochemistry & Molecular Biology, the University, Manchester M13 9PT** by June 1st, 1989. The University is an equal opportunities employer. (9077)A

**UNIVERSITY OF EXETER DEPARTMENT OF BIOLOGICAL SCIENCES**

**RESEARCH FELLOW (PLANT PHYSIOLOGIST)**

Applications are invited for the above post for research into the mechanisms of Arsenic tolerance in the grass *Holcus lanatus*. The project will involve the study of the uptake of arsenate and phosphate and their interaction. The position will commence on June 1st, or as soon as possible thereafter for a period of three years. Starting salary £9865 (under review).

Applications with the names of two referees, should be sent to **Dr M R Macnair, Hatherly Laboratories, Department of Biological Sciences, University of Exeter, by May 16th**, from whom further particulars may be obtained. (9079)A

**POSTDOCTORAL POSITION**

**MOLECULAR NEUROBIOLOGY**

Immediate opening on NIH grants to study the neurotrophic effects of the insulin-like growth factor gene family, nerve regeneration, synaptogenesis, or pathogenesis of diabetic neuropathy. Location near scenic Rocky Mountains. Submit curriculum vitae with names and phone numbers of three references to: **Dr. Douglas Ishii, Physiology Department, Colorado State University, Fort Collins, CO 80523.** CSU is an Equal Opportunity/Affirmative Action Employer (NW3667)A

**MICROBIOLOGIST/IMMUNOLOGIST**

A full-time tenure track position is available in the Faculty of Dentistry's Department of Oral Biology with a joint appointment in the Faculty of Medicine's Department of Microbiology. Candidates must possess doctoral level education in microbiology, immunology, or a related field of study. Teaching and research experience in the microbiological and/or immunological aspects of oral diseases is preferred. Responsibilities include course administration and teaching of oral microbiology and immunology to dental, dental hygiene and graduate students. Collaborative research with dental and dental hygiene faculty members is strongly encouraged. Rank and salary will be commensurate with qualifications and experience. Interested applicants should send Curriculum Vitae and three reference letters by 30 June 1989 to **Dr. R. Howell, Acting Chair, Department of Oral Biology, Faculty of Dentistry, Dalhousie University, Halifax, Nova Scotia B3H 3J5.** In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents.

Dalhousie University has a policy of affirmative action in hiring qualified women academic staff. (NW3657)A

**ASSISTANT PROFESSOR/INSTRUCTOR/STAFF ASSOCIATE.**

Available immediately to join the Immunogenetics Laboratory with interest/experience in HLA and/or lymphocyte differentiation antigens.

Requirements: Ph.D. and 3 years of postdoctoral training in molecular genetics.

Send curriculum vitae and bibliography to:

**Dr. Nicole Suciu-Foca, College of Physicians and Surgeons of Columbia University, Department of Pathology, Dept. NT P & S 14-403, 630 West 168th Street, New York, NY 10032.**

(NW3638)A

**Two Postdoctoral Positions in Protein Crystallography and Engineering**

One position is for a macromolecular crystallographer to work on cytochrome P-450 and related systems. The second is for a biochemist/biochemist interested in working with molecular biologists and crystallographers on heme enzyme protein engineering. Contact

**Dr. T.L. Poulos, University of Maryland's Center for Advanced Research in Biotechnology, 9600 Gudelsky Drive, Rockville, Maryland 20850.**

(NW3643)A

**THE UNIVERSITY OF LEEDS DEPARTMENT OF PHARMACOLOGY**

**POSTDOCTORAL RESEARCH FELLOW**

Applications are invited for the above post for work on an MRC co-operative project investigating the protective effects of adenosine antagonists in acute renal failure.

Candidates must possess a PhD or relevant experience. The appointment is for one year.

Salary within the range £9865-£11070 on the IA Grade for R/A staff according to qualifications and relevant experience.

Informal enquiries may be made to either **Dr C J Bowmer** or **Dr M S Yates** (tel (0532) 334315/6).

Application forms and further particulars may be obtained from and completed applications forwarded to the Registrar, the University, Leeds LS2 9JT, quoting reference no. 103/13. Closing date for applications 18 May 1989. (9122)A

**POSTDOCTORAL RESEARCH ASSOCIATE**

**Mechanism of HIV Cytopathicity**

Position available to participate in studies on mechanism in HIV mediated cytopathicity and mechanisms of viral persistence in AIDS (Stevenson et al, Cell 53: 483-496, 1988). Experience in molecular biology is desirable. C.V. & 3 letters of recommendation to **Mario Stevenson, Ph.D., Pathology & Microbiology, University of Nebraska Medical Center, 42nd & Dewey Ave., Omaha, NE 68105** by 8-1-89.

An Affirmative Action/Equal Opportunity Employer.

(NW3645)A

**POST DOCTORAL RESEARCH ASSOCIATES**

Positions are available for candidates with experience in smooth muscle physiology, biochemistry, cell and molecular biology to work on eicosanoids regulation of human myometrial functions. Some experience in eicosanoids research would be helpful. Address inquiries to **Dr. Ch. V. Rao, Dept. of Ob/Gyn, 438 MDR Bldg., University of Louisville, Louisville, KY 40292.** (NW3639)A

**POST-DOCTORAL POSITIONS**

available immediately to work on the molecular biology and immunology of the parasites that cause the major tropical diseases. Send resume and three letters of reference to **Dr. John E. Donelson, Dept. of Biochemistry, University of Iowa, Iowa City, IA 52242 USA.**

The University of Iowa is an EO/AA employer. (NW3640)A

## UNIVERSITY OF MINNESOTA ASSISTANT PROFESSOR

The University of Minnesota invites applications for one full-time position in the field of human population genetics at the rank of assistant professor (tenure-track). The position will have a primary appointment in the Department of Laboratory Medicine and Pathology and a joint appointment in the Institute of Human Genetics. Candidates with a Ph.D. and/or M.D. with advanced training in population genetics or related disciplines, plus 2 years of relevant post-doctoral experience that includes human gene mapping or complex segregation analysis will be considered. Candidates will be expected to develop a vigorous, independent research program; participate in teaching; and interact with Institute members in the development of the Institute. Requirements: In-depth knowledge of multilocus linkage analysis; complex segregation analysis and/or evolution at the molecular level. Please send curriculum vitae; a short description of research plans; and three letters of recommendation to: **Chair, Search Committee, Institute of Human Genetics, University of Minnesota Medical School, Box 206(N), UMHC, Harvard Street at East River Road, Minneapolis, MN 55455.** The last date for receipt of applications is 31 July 1989. *The University of Minnesota is an Equal Opportunity Educator and Employer and specifically invites and encourages applications from women and minorities.*

(NW3663)A

## Molecular Biology Growth Regulation

The Division of Hematology-Oncology, Department of Pediatrics at Childrens Hospital of Los Angeles, and the Departments of Pediatrics and Biochemistry, of the University of Southern California School of Medicine are seeking an established Molecular Biologist whose investigation is in the area of growth regulation of normal and malignant hematopoietic cells. This investigator should be able to develop and direct an outstanding laboratory program as well as interact with individuals performing clinical/laboratory investigations. The job profile includes teaching in hematology-oncology and biochemistry (20%), and laboratory investigation (80%).

The candidate must have a proven ability to obtain peer-received grant research funding. Substantial institutional support is available for this individual.

Interested candidates should send a curriculum vitae and list of three references to: **Dr. Robert C. Seeger, M.D., Division of Hematology-Oncology, Childrens Hospital of Los Angeles, 4650 Sunset Boulevard, Los Angeles, California 90027.**

The University of Southern California and Children's Hospital of Los Angeles are Equal Opportunity Employers.

(NW3675)A

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At BERLEX Laboratories, a growing leader in the pharmaceutical industry, our commitment to R & D has never been stronger. As a result, we're currently seeking Chemists who want to maximize their talents in our progressive scientific environment.

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In addition to the constant challenge of discovery, we provide a generous salary and benefits package including comprehensive medical & dental coverage and 100% tuition reimbursement, with ample opportunity for growth. For consideration, send your resume with salary history and requirements to: **MARILYN HUNDLEY, Manager Human Resource Operations, BERLEX LABORATORIES, INC., 300 Fairfield Road, Wayne, New Jersey 07470.** Equal Opportunity Employer M/F.

NW(3649)A

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## Regulatory Toxicologists (Senior and Postdoctoral)

The Agricultural Research Division of American Cyanamid Company has a long-standing and highly successful commitment to the discovery and development of products to improve worldwide crop and livestock production. We are growing rapidly and currently have the following opportunities at our laboratory and experimental farm complex ideally located near Princeton, one hour from New York City and Philadelphia.

### Senior Toxicology Program Manager

The successful candidate will have a PhD in Toxicology or related medical field and 3 to 5 years of industrial, government or contract laboratory experience in toxicology. Knowledge of whole animal toxicology is essential. You will design, monitor and review the full range of toxicity testing performed in-house or on-contract for EPA and FDA registrations of pesticides and animal drugs.

### Postdoctoral in Regulatory Toxicology

We offer a postdoctoral position which requires a PhD in Toxicology or related medical field.

American Cyanamid offers a competitive compensation and benefits package. For prompt and confidential consideration, please forward your resume and salary history, indicating the position of your specific interest, to the Employment Office, Dept. NA-427, American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540. An Equal Opportunity Employer, M/F/H/V.



THE COMMITMENT GROWS EVERY DAY.

NW(3655)A



# UNIVERSITY OF NATAL

PIETERMARITZBURG,  
SOUTH AFRICA

The University of Natal rejects apartheid. It is an equal opportunity, affirmative action University.

## Department of Microbiology and Plant Pathology

### Professor in Microbiology

Ref. P27/89

Closing date: 9 June 1989

A doctorate in Microbiology is essential. Research experience in aspects of modern industrial microbiology or biotechnology will be a strong recommendation.

Applicants must be able to provide academic leadership and should have a proven record of achievement in teaching, postgraduate supervision and research.

Current research interests in the sub-Department centres around industrial microbiology, especially the utilisation of agricultural waste materials.

The salary offered will be determined according to the qualifications and/or experience of the successful applicant.

Application forms, details of the salary package, further particulars of the post and conditions of service are obtainable from the Personnel Section University of Natal, P O Box 375 Pietermaritzburg, 3200, South Africa, telephone (0331) 63320 or The Secretary, South African Universities Office, Second Floor, 16 Charles II Street, London SW1Y 4QU. Telephone 01-839 3420 or 01-839 4388. Fax 01-839 3480. (W6087)A

## UNIVERSITY OF BRISTOL DEPARTMENT OF MICROBIOLOGY

Applications are invited for a

### Research Assistant 1B

post to be taken up as soon as possible. The appointment ends 30th December 1991.

We are studying the survival of the bacterium *Rhizobium* in Italian soils as part of a collaborative project with scientists in Ireland and Italy.

We will set up database of information about important characteristics of the rhizobia and environment from which they are isolated, to correlate survival with particular environmental features, such as soil pH or cropping history.

The position is suitable for a graduate in biology with some experience of IBM-compatible personal computers. Training will be given to a particularly well qualified biologist, if needed.

Informal enquiries may be made to Professor J E Beringer on Bristol (0272) 303759.

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference A368.

An Equal Opportunities employer.

(9076)A

## ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

### POST-DOCTORAL RESEARCH FELLOW

required for a 3 year project involving the development of human retroviruses as eukaryotic gene vectors, and evaluation of viral packaging mechanisms. Technical support will be provided. Excellent facilities available. A background in molecular biology is required.

Salary up to £11,680 plus £1,650 London allowance in scale 1A for University Research Staff (under review). Further details and an application form from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, 01-672 9944 ext 56020. Closing date 19 May 1989. Please quote reference 55/89. (9092)A

## The Wellcome Trust/ Cancer Research Campaign Institute of Cancer and Developmental Biology

UNIVERSITY OF CAMBRIDGE, ENGLAND

This new research Institute is now under construction in the Science area of Cambridge University. It will provide excellent facilities for some independent research groups, each funded by one of the above sponsors. We are now seeking outstanding young scientists, (currently of post-doctoral level or above) as leaders of independent groups.

The first appointments can be made this summer to be taken up when the Institute opens at the end of 1990. Appointments will be made at the Wellcome/CRC Senior Fellowship level and will be for five years in the first instance. Appointments will include funding for a small group and research expenses.

For further information on the current staff composition, aims and interests of the Institute, please write to J.B. Gurdon (Chairman), CRC Molecular Embryology Unit, Department of Zoology, Downing Street, Cambridge CB2 3EJ. Telephone (0223) 323316.

The University follows an equal opportunities policy. (9096)A

## Senior Technician Grade 8C (Molecular Biology/Genetics and Biochemistry)

Salary Scale £13,575 - £14,736 inclusive of London Allowance

This is a newly created post in the School of Biological and Health Sciences responsible for providing a state-of-the-art service and contributing to the development of improved methods of experimental biochemistry, gene manipulation and molecular biology. Applicants should have a degree or equivalent qualification and relevant experience.

Please telephone 01-580 2020 ext 2136 (answerphone) for an application form and further details or write to Personnel Department, PCL, 309 Regent Street, London W1R 8AL quoting Ref 10402. Closing date for the receipt of completed application forms is 18 May 1989.

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OF CENTRAL LONDON

(9132)A



ROYAL HOLLOWAY AND BEDFORD  
**NEW COLLEGE**  
University of London

## CELL BIOLOGY LABORATORY RESEARCH ASSISTANT

required to join an expanding team led by Professor Michael O'Shea investigating the molecular biology and biochemistry of peptide hormones in insects.

The successful applicant will participate in studies of the structure and biosynthesis of precursor proteins of insect neuropeptide hormones. A degree in Biochemistry, Molecular Biology or a related discipline is required and Postdoctoral applicants with suitable experience are encouraged to apply. Experience with DNA sequencing and cloning methodologies would be an asset. Applicants with experience of protein and peptide purification and protein chemistry will also be considered.

Applicants may telephone Professor O'Shea on 0784 39970 to discuss the specifics of the research project and to arrange an informal visit to the laboratory, if required.

Application forms may be obtained from the Personnel Office, RHBNC, Egham Hill, Egham, Surrey. TW20 0EX. Closing date for receipt of applications is 19th May 1989. (9118)A



## OCEAN DRILLING PROGRAM TEXAS A&M UNIVERSITY STAFF SCIENTIST

The Ocean Drilling Program at Texas A&M University invites applications for the position of Staff Scientist in the Department of Science Operations. Applicants must have a Ph.D. in geology or related field and a strong background in marine geology. Experience as a seagoing scientist is preferred. Applicants must demonstrate fluency in written and spoken English. Responsibilities include sailing on a scientific research drillship as staff scientific representative approximately once per year, and coordinating post-cruise scientific research and publication of cruise results. Individual research and interaction with colleagues in the Departments of Oceanography, Geology, and Geophysics are encouraged.

Salary is commensurate with qualifications and experience. Closing date for submitting applications is June 15, 1989. Applicants should forward curriculum vitae, bibliography, statement of research interests, and names and addresses of four (4) references to:

**Employment Manager  
Personnel Department  
Texas A&M University  
YMCA Building  
College Station, Texas 77843**  
Equal Opportunity Employer  
(NW3666)A

# SCIENTISTS CARDIOVASCULAR RESEARCH

**COR Therapeutics, Inc.** is a recently established and rapidly growing biotechnology company located in the San Francisco Bay Area. We specialize in the development of novel therapeutics for cardiovascular disease. We are actively recruiting a few highly qualified scientists with proven track records to join our Research Department. These scientists will join a research effort directed at the study of molecular mechanisms underlying various aspects of cardiovascular disease. The primary focus will be on growth factors and growth factor receptors, and receptors mediating vascular cell interactions.

### Molecular Biology

Expression of native, chimeric and mutant proteins. Experience with mammalian cell expression preferred. Objectives will be to use a molecular approach to understand protein function and to develop the use of expressed proteins as novel pharmaceutical agents.

**COR Therapeutics, Inc.** offers competitive salaries, benefits, and attractive equity positions to its employees combined with the challenge and opportunity to make a significant contribution to this new organization. To apply, please send *curriculum vitae* to COR Therapeutics, Inc., 256 E. Grand Avenue, Suite 80, South San Francisco, CA 94080, ATTN: Human Resources. EOE. (NW3665)A

### Protein Biochemistry

Identification, isolation and characterization of structural and functional domains of ligands and receptors. Objectives will be to characterize structure-function relationships of these proteins and to use this information to develop receptor antagonists.

## COR Therapeutics, Inc.

## Postdoctoral Positions

### Harvard Medical School Dana Farber Cancer Institute and Joint Center for Radiation Therapy

A number of postdoctoral positions are available to study the malignant phenotype and its influence on responses to cancer therapy. Areas of interest include: regulation of the heat shock response and functions of heat shock proteins; effects of hypoxia on gene expression in malignant cells; role of cellular redox state in responses to cancer therapy and in pleiotropic drug resistance; growth factors and the control of hematopoietic cell proliferation; phenotypes in human mammary cancer — cell surface glycoproteins, tyrosine phosphoproteins, steroid receptors and oncogene expression; antibody-targeted cancer therapy. Applicants should have a strong training in cell biology, molecular biology or biochemistry. Applications, giving full curriculum vitae and the names of two referees to **Dr. Stuart Calderwood, Dana Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115.** (NW3669)A

## PHYSICIST

Assistant Professor, tenure-track, recent Ph.D., with background in electron optical research, particularly electron energy loss spectroscopy and interest in biological applications. Opportunity also for participating in research involving confocal microscopy and microspectrophotometry. Appointment is to be made in the Department of Physiology; the Appointee will be encouraged to develop and maintain ties with the Department of Physics and/or Materials Science. The position is suited for individuals desirous of developing an independent research program as well as collaborating with cellular biophysicists in the development and application of electron and light optical methods to problems of cell biophysics. A good grasp of theory, as well as instrumentation, is required. The University of Virginia is an Equal Opportunity Employer. Please furnish curriculum vitae and résumé to: Dr. A.P. Somlyo, Chairman, Department of Physiology, Box 449, University of Virginia, Charlottesville, VA 22908. (NW3671)A

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Replies as soon as possible with full *curriculum vitae* to the Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF. (9061)A

## POSTDOCTORAL POSITION

Available immediately to participate in research directed toward a molecular understanding of cell differentiation and reproduction in the filamentous fungus *Aspergillus nidulans*. Classical and molecular genetic approaches will be used to investigate the expression of essential genes and biochemical functions of gene products. Experience in molecular biology or genetics is desirable.

Send résumé and three letters of reference to: Dr. Bruce L. Miller, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843. Equal Opportunity/Affirmative Action Employer. (NW3674)A

## UNIVERSITY OF WARWICK HEAD TECHNICIAN HIV RESEARCH

The Virology Group within the Department of Biological Sciences has received a large grant from the AIDS Directed Programme of the Medical Research Council to begin on HIV. A new suite of containment laboratories will be constructed to allow the work to proceed and we are seeking to appoint a senior technician for this facility. He/she will have day to day responsibility for the running of the laboratories in addition to assisting in ongoing research projects. Applicants with experience in handling infectious agents are particularly encouraged to apply but additional training in the special requirements for handling HIV will be given.

The appointment is for three years in the first instance. Salary on the Grade 5 scale: £8088-£9549.

Informal telephone enquiries concerning this post can be made to Dr M A McCrae on 0203-523524.

Application forms from the **Personnel Office, University of Warwick, Coventry CV4 7AL** (0203 523685) quoting Ref No 35/T/88 (please mark clearly on envelope).

Closing date for applications 15 May 1989.

**AN EQUAL OPPORTUNITIES EMPLOYER (9086)A**



ROYAL HOLLOWAY AND BEDFORD  
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University of London

### DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the position of

## Postdoctoral Research Assistant

to work with Dr C C Rider on the binding of lymphokines by the glycosaminoglycans of lymphoma cells and lymphocytes. Glycosaminoglycans are long, linear chains of carbohydrate, and they exhibit structural differences between the B and T cell lineages. This project is funded by the leukaemia Research Fund for 2 years and is available as soon as possible.

Applicants should possess or be expecting to gain a PhD in Biochemistry, Immunology or Cell Biology.

The work will involve fractionation of the glycosaminoglycans synthesized by lymphoma cell lines and the determination of lymphokines by bioassay or immunoassay.

Salary on the scale £11,515 - £17,370 a year including London allowance. Informal enquiries may be made to Dr C C Rider, Department of Biochemistry, (0784 34455 ext. 3548).

Application forms are available from the **Personnel Office, RHBNC, Egham Hill, Egham, Surrey. TW20 0EX. Completed applications should be returned by 17th May 1989.** (9071)A

## POSTDOCTORAL POSITION

Study the cellular basis for auditory information processing and synaptic organization of auditory brainstem. Use axonal transport, EM autoradiography, EM immunocytochemistry, electrophysiology, and intracellular staining. Participate in high-tech program to use image processing and 3D-computer graphics in neuroanatomy. Ph.D. in neurobiology or equivalent required plus experience in neuroanatomy, EM, or neurophysiology. Join an exciting interdisciplinary group.

To apply, send curriculum vitae and reference to: **Dr. Douglas L. Oliver, Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.**

*An Affirmative Action/Equal Opportunity Employer M/W/H.* (NW3641)A

## BOTANIST

Bishop Museum is seeking an assistant/associate botanist to conduct original systematic and evolutionary research on Hawaiian and tropical Pacific flowering plants and to provide expertise on Hawaiian plants to the scientific community and public. This is a Bishop Museum-funded position. Candidates must have a PhD degree and preferably some experience with floristic botany. Send curriculum vitae and letter of application to: Research Botanist Search Committee, Bishop Museum, PO Box 19000-A, Honolulu, HI 96817-0916. An Equal Opportunity Employer. (NW3672)A

## The DEPARTMENT OF PATHOLOGY of the UNIVERSITY OF MONTREAL Medical School invites applications for the following faculty position:

A) Associate or full professor M.D. and/or Ph.D. with experience in research in oncology and molecular biology. The selected candidate will be expected to act as the scientific coordinator of a developing group in oncogenetic research and to share teaching responsibilities, mostly at the graduate level. A good knowledge of the french language is preferable. Salary will be commensurate with academic level.

B) Research professor M.D. and/or Ph.D. interested in developing research in molecular genetic or molecular biology. The candidate will integrate a research group in oncogenetics and will share responsibilities in teaching at all levels. The candidate's profile should allow competitiveness for the obtention of external research funds in the two years following his or her arrival. A good knowledge of the french language is preferable.

Send curriculum vitae and the names of three referees by June 15, 1989 to: **Dr. Jean Michaud, Chairman, Department of Pathology, Université de Montreal, P.O. Box 6128, Station "A", Montreal, Quebec, H3C 307.**

In accordance with Canadian Immigration requirements, this advertisement is directed in priority to Canadian citizens and permanent residents of Canada. The University of Montreal is an equal opportunity employer.

(NW3664)A

## CITY & HACKNEY HEALTH AUTHORITY

QUEEN ELIZABETH  
HOSPITAL FOR CHILDREN,  
HACKNEY ROAD,  
LONDON, E2 8PS

"Working towards Equal Opportunities"

Applications are invited for the post of MLSO 1 in the Academic Department of Child Health Laboratories at Queen Elizabeth Hospital for Children, Hackney Road, London, E2 8PS. The successful applicant will assist with molecular biology techniques in relation to IgE synthesis in young atopics. Experience of RNA handling, tissue culture and immunoassays, particularly ELISA, desirable but not essential. Appointment initially for one year with the possibility of renewal. Salary £10,543 to £10,917 per annum.

Applications to be sent to: Joint Academic Department of Child Health at the above address, as soon as possible. (9109)A

The University of Queensland  
Brisbane, Australia

Lecturer/Senior Lecturer (Fixed Term)

Department of Physiology and Pharmacology

With higher degree and active research interests and experience in Central Nervous System (other than Sensory Neuroscience) or Muscle. Applicants should have demonstrated ability to contribute to the Department's teaching programs to students in the Faculties of Medicine, Science, Veterinary Science, Dentistry, Education and Agriculture. Appointment available from 1 January 1990.

Salary: Lecturer A\$30,737 - A\$40,100 per annum; Senior Lecturer A\$40,937 - A\$47,564 per annum.

Closing date: 16 May 1989. Reference No: 18489.

Additional information available from Professor Elspeth McLachlan (07) 377 3133, or from Appointments (36278), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Equal Opportunity in Employment is University Policy (W6088)A

## POSTDOCTORAL POSITION SUNY STONY BROOK

Funded project for a postdoctoral to become immediately productive using available reagents and probes to study the expression of specific enzymes that control normal and malignant cell behaviour. Experience in molecular biological techniques essential. \$25,000 stipend. Send CV and references to:

**Dr. James P. Quigley, Department of Molecular and Cellular Pathology, SUNY/Stony Brook, Stony Brook, NY 11794-8691.**

*SUNY/Stony Brook is an affirmative action equal opportunity educator and employer.*

(NW3651)A

## POSTDOCTORAL AND RESEARCH ASSISTANT POSITIONS

available immediately to study immunotherapeutic approaches to treatment of experimental metastases in a mouse model. Postdoctoral applicant should have an M.D. and/or Ph.D. with a strong background in tumor immunology. Send curriculum vitae and names of at least two references to: **Dr. Jerry A. Bash, Surgical Cancer Research Laboratory, Department of Research, Mount Sinai Medical Center, 4300 Alton Road, Miami Beach, FL 33140.** (NW3637)A

**University of York  
Department of Biology**

**Postdoctoral Research Fellow**

Applications are invited for the above AFRC-funded post, available from June for a period of up to two years and two months. The project involves ion-selective microelectrode measurements on plant cells, with the aim of elucidating the role of cytosolic free calcium in growth and development. The successful candidate will join a well-equipped laboratory active in various aspects of calcium metabolism.

Starting salary within the range £9,865-£11,680 per annum.

Two copies of applications, with full curriculum vitae and naming two referees, should be sent by 16 May 1989 to Personnel Office, University of York, Heslington, York YO1 5DD, from whom further particulars are available. Please quote reference number 6/6273A.

Informal enquiries to Dr D Sanders (tel. 0904 432825) (9082)A

**UNIVERSITY OF LIVERPOOL  
DEPARTMENT OF BIOCHEMISTRY  
RESEARCH ASSISTANT**

Applications are invited from suitably qualified graduates or intending graduates in biochemistry or a related subject to work for Professor B. E. H. Maden on a grant from the Wellcome Trust for research into human ribosomal genes. The work is to explore the application of some new techniques such as genomic sequencing and the polymerase chain reaction. The post is tenable for twelve months with the possibility of renewal.

Salary within the range £8,675-£9,865 per annum.

Applications, together with the names of three referees, should be received not later than 12 May 1989, by The Director of Staffing Services, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained.

Quote ref. RV/334/N.

An Equal Opportunity Employer.

(9081)A

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**RESEARCH FELLOW  
Molecular Biology**

\$A31,525-\$A38,567

DIVISION OF ANIMAL PRODUCTION  
PROSPECT, NSW, AUSTRALIA.

THE DIVISION: The Division of Animal Production conducts research directed towards the general improvement in production efficiency of domestic animals. The research encompasses a wide area, including genetics, nutrition, reproduction, physiology, skin biology and immunobiology.

THE PROGRAM: One of the major programs of the Division has as its aim the genetic engineering of animals. The techniques required for the production of transgenic livestock have been established and the research is now involved in understanding the regulatory mechanisms which control foreign gene expression in transgenic animals.

THE JOB: The appointee will undertake research with a team involved in the genetic engineering of sheep and will participate in an investigation of the fundamental mechanisms which control gene expression in transgenic animals. Particular emphasis will be placed on the tissue-specific regulation of genes which have been constructed in order to introduce new metabolic pathways into transgenic sheep.

THE PERSON: Applicants should hold a PhD degree or equivalent qualifications with research achievement in biochemistry and molecular biology. Practical experience in recombinant DNA techniques is essential. Familiarity with cell culture techniques and protein chemistry would be an advantage.

CONDITIONS: Appointment will be for a term of 3 years, with Australian Government Superannuation benefits available.

MORE INFORMATION: Prospective applicants are invited to telephone Dr Kevin Ward on (02) 631 8022 for further information. Dr Ward can also provide a copy of the detailed job description and selection criteria.

APPLICATIONS: Applications should be submitted by 25th May, 1989, and quote reference No. AP89/16. They should be framed against the selection criteria, and should state relevant personal particulars including details of qualifications and experience. Applicants should nominate at least two professional referees, and address their applications to:

The Chief,  
CSIRO Division of Animal Production,  
P.O. Box 239, Blacktown, N.S.W. 2148,  
Australia.



CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W6084)A

**ASSISTANT PROFESSOR, MICROBIOLOGY**

The Department of Microbiology, Faculty of Medicine, Dalhousie University seeks a Ph.D. with a strong background in Microbiology and postdoctoral training preferably in Molecular/Tumor Virology to establish an independent research program and participate enthusiastically in undergraduate and graduate teaching programs. Specialists in other areas may also be considered. Interested applicants should send Curriculum Vitae, three letters of reference, and an outline of their proposed research program as soon as possible to Dr. K.B. Easterbrook, Head, Department of Microbiology, Dalhousie University, Halifax, Nova Scotia B3H 4H7. It is expected that this will be a tenure-track position and it will remain open until filled. In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents. Dalhousie University has a policy of affirmative action in hiring qualified women academic staff. (NW3656)A

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**FACULTY POSITION  
THERIOGENOLOGY: LARGE ANIMAL OR WILDLIFE**

**Position Description**

The Department of Clinical Sciences of the New York State College of Veterinary Medicine invites applications for a tenure track position in Theriogenology with responsibilities in large animal theriogenology or wildlife reproduction. The ideal candidate should possess a DVM degree or equivalent and have post-graduate research training and experience. Preference will be given to an individual with demonstrated expertise and interest in equine theriogenology or in reproduction of wildlife or endangered species. Board certification or board eligibility in the American College of Theriogenologists is highly desirable. The Theriogenology Section at Cornell currently includes five faculty positions in large and small animal theriogenology. The successful candidate will participate in the large animal clinical theriogenology service in the Veterinary Medical Teaching Hospital and the teaching of formal courses in the professional (DVM) core curriculum. The candidate will be required to develop a high quality, independent research program, and to participate in professional service and continuing education activities. Opportunities for collaborative research are excellent.

Rank and salary will be commensurate with professional and academic credentials and experience. Interested individuals should send a letter of application, curriculum vitae and names and addresses of three suitable referees to **Dr. Donald F. Smith, Chairman, Department of Clinical Sciences, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401.**

Cornell University is an equal opportunity/affirmative action employer/educator. (NW3668)A

**University of Sheffield**

**CHAIR OF ORGANIC CHEMISTRY**

Applications are invited for the Chair of Organic Chemistry which will become vacant on 1 October 1990 following the retirement of Professor W D Ollis FRS.

Further particulars from the **Director of Personnel Services, The University, Sheffield S10 2TN** to whom applications (1 copy), including a full curriculum vitae, a list of publications with page numbers and the names and addresses of three referees should be sent by 9 June 1989. Please quote reference number R.883.

An Equal Opportunity Employer

(9083)A



**at the leading edge**

**THE LONDON HOSPITAL MEDICAL COLLEGE  
(University of London)**

**MOLECULAR BIOLOGIST**

Applications are invited for a research assistant position to work on the molecular biology of mycobacterial heat-shock proteins (Nucleic Acids Res. (1988) 16, 9047, J. Gen. Mic. (1989)) (in press).

Experience in expression of recombinant proteins would be an advantage. The project is located in the Department of Medical Microbiology which is well equipped for this type of work and contains several groups engaged in molecular studies. If the successful candidate does not have a PhD and wishes to take a higher degree, they will be particularly encouraged.

For further details, telephone Dr A R M Coates on 01-377 7643. A curriculum vitae with names and addresses of two referees should be sent to **Dr A R M Coates, Dept of Medical Microbiology, London Hospital Medical College, Turner Street, London E1 2AD.**

(9095)A

**THE HANNAH RESEARCH  
INSTITUTE  
DIRECTOR'S RESEARCH  
GROUP  
IMMUNOLOGIST/  
BIOCHEMIST**

Applications are invited for a position in the Director's Research Group to study the autocrine control of milk secretion. The appointee will join a vigorous team which is investigating local modulation of the endocrine control of mammary development and function by autocrine and paracrine mechanisms operating within the gland. The post is funded for 2 years and is available immediately.

Applicants should possess a first or upper second class degree in biochemistry, immunology, or a related discipline, with at least 2 years' relevant experience. Experience in the production of monoclonal antibodies and in radio-immunoassay or enzyme immunoassay development is desirable but not essential.

Appointment will be at Higher Scientific Officer grade, with starting salary up to £10,994. A non-contributory superannuation scheme is operating.

Further details may be obtained from the Secretary, The Hannah Research Institute, AYR KA6 5HL to whom applications, including the names of two referees, should be forwarded by Friday 19 May quoting reference HRI/165. (9114)A

**POSTDOCTORAL  
POSITION**

**Molecular Enzymology  
and Protein Engineering**

Available immediately to study the molecular cloning, mutagenesis and chemical mechanism of action of mammalian phosphatases. Experience with  $\lambda$ , cloning and DNA sequencing is essential. Send curriculum vitae and names of three references to: **Prof. Robert L. Van Etten, Chemistry Department, Purdue University, West Lafayette, IN 47907. Purdue University is an Equal Opportunity/Affirmative Action Employer. (NW3654)A**

**POSTDOCTORAL POSITIONS**

available to conduct research on RFLP analysis of corn and other cereal crops. Applicants should have experience with standard molecular biology techniques. Familiarity with DNA sequencing and PCR technology is desirable. Candidates with a PhD in molecular biology and/or genetics are encouraged to apply. Please send résumé and transcripts to: Sybil Paul, Department of Agronomy, Virginia Polytechnic Institute and State University, Blacksburg, VA24061. Telephone: 703/231-6300. VPI&SU is an Equal Opportunity/Affirmative Action Employer. (NW3670)A

**CYTOGENETICIST MD/Ph.D.**

Physician or pathologist preferred. Specialty certification (or equivalent training) and background in clinical diagnostic and research work important. Funded basic research grant support and evidence of productivity critical. Experience and a record of scholarship with a strong publication record are required. Minimum of two years experience in human cytogenetics necessary. Women and members of minority groups are encouraged to apply. Please forward curriculum vitae to **John E. Craighead, M.D., Department of Pathology, University of Vermont, Burlington, VT 05405.** Applications will be accepted until position is filled. Affirmative Action/Equal Opportunity Employer. (NW3644)A

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## INSECT NEUROBIOLOGIST

Full-time tenure-track appointment at the rank of assistant professor. We seek candidates having research interests in areas such as: integrative functions of the insect CNS, neuroendocrine mechanisms, neurogenetics, neurodevelopment, neuron physiology and pharmacology. Appointee will teach an undergraduate course in general insect physiology and conduct graduate seminars. Applicants should have a Ph.D. and should demonstrate outstanding potential for research and teaching.

Applications should include the following: (1) Curriculum vitae, academic transcripts and evidence of teaching skills and experience; (2) bibliography and selected reprints or manuscripts; (3) statement of research objectives and approaches proposed; (4) names and addresses of at least three people who can provide recommendations. Submit all materials by 1 August 1989 to **D.L. Wood, Chairman, Department of Entomological Sciences, 201 Wellman Hall, University of California, Berkeley, Calif. 94720.**

*The University of California is an Equal Opportunity, Affirmative Action Employer.* (NW3673)A

## FELLOWSHIPS

# Postdoctoral Fellowship

Applications are invited for a Postdoctoral Research Fellowship to participate in a collaborative programme between the Imperial Cancer Research Fund, Oxford University and the MRC Radiobiology Unit.

The work involves NMR and biochemical studies on experimental and human tumours, and the person appointed should have some experience in NMR or an appropriate background in biochemistry with an interest in NMR in vivo.

The appointment is for three years and is on a salary scale £12,500–£16,000 depending on age.

Applications should be made by sending a full cv, including the names and addresses of three referees, to **Professor G.K. Radda, FRS, Department of Biochemistry, Oxford University, South Parks Road, Oxford OX1 3QU**, from whom further particulars may be obtained. Tel no: 0865-275274. Fax: 0865-275259.

(9064)E

**I M P E R I A L  
CANCER RESEARCH FUND**

## ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London) JUNIOR RESEARCH FELLOWSHIPS

Applications are invited for six Fellowships from basic science students (in appropriate disciplines) who have, or expect to obtain, first or upper second class degrees and are highly motivated towards research. The conditions of the Fellowships are similar to SERC Ph.D. Research Studentships and are for 3 years from October 1989. They provide a maintenance grant of £3970 per annum. The supervisors and research topics are:

**Dr S.A. Baldwin** (Depts of Biochemistry and Protein & Molecular Biology) **Dr D.W. Wray** (Dept. of Pharmacology)

"Structure, Function and Regulation of a Voltage-Dependent Calcium Channel"

**Prof D. Chapman, FRS** (Dept of Protein & Molecular Biology)

"Protein Folding and Polymerization Processes. Studies of RNAaseT, Mutants and Fibrinogen Using FTIR Spectroscopy"

**Prof B.A. Cooke and Mr S. Parbhoo** (Molecular Endocrinology Group)

"Genetic Expression and Activity of Aromatase in Benign and Malignant Breast Tissue"

**Dr T. Cowen** (Dept of Anatomy)

"Neuronal Ageing: A Study of the Axonal and Dendritic Arborisation of Transmitter-Identified Autonomic Neurons in Old Age"

**Dr E.S. Debnam & Dr S. K. Srai** (Dept of Physiology)

"Isolation and Characterisation of Brush Border Membrane Iron Binding Protein and its Expression in the Developing Guinea Pig"

**Dr M. Jacobs** (Dept of Pharmacology)

"Endothelium-Derived Vaso-Active Factors, Lipoproteins and Atherosclerosis"

Further details from and applications (which should be made as soon as possible) to **The Registrar, The Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, Tel 01-794 0500 ext 4258.** (9104)E

## The Queen's University of Belfast POSTDOCTORAL FELLOWSHIPS School of Biology and Biochemistry

A number of fellowships will be available for one to three year periods in the following areas: Biochemistry — (peptide, nucleic acid and immunochemistry), Genetic Engineering — (virology, oncology, microbial, plant molecular biology), Cell and Experimental and Evolutionary Biology — (behaviour, ecology, fish biology and marine biology).

Salary scale (under review): £9,867–£15,720 per annum, with USS, placing depending on age and experience, with USS.

Further information about the School's research programme may be obtained from **Professor S.J. Martin (Belfast (0232) 329241 ext. 2491).**

Applicants, quoting ref. 89/BB, should submit a curriculum vitae, including the names and addresses of three referees to the **Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland.** Closing date: 28 May 1989. *The University is an Equal Opportunity Employer.* FEA Registration no. 575. (9115)E

continued on page 32

## STUDENTSIPS



## CENTRAL TOXICOLOGY LABORATORY PhD STUDENTSHIP IN TOXICOLOGY

To work in the Biochemical Toxicology Section to study mechanisms of nephrotoxicity. The student will join a thriving research environment with opportunities to collaborate with other groups in ICI. The project will investigate the metabolic activation of chemicals and their subsequent toxic effect in renal cells. Techniques to be used include cell isolation and culture, enzyme kinetics, identification of metabolites, radiotracer studies and immunocytochemistry. Prospective students should expect to receive a 1st or Upper 2nd class honours degree in biochemistry or a related subject. ICI offers excellent laboratory facilities and will supplement the standard studentship grant with a local living allowance.

Further information from **Dr E A Lock, Imperial Chemical Industries, Central Toxicology Laboratory, Alderley Park, Cheshire, SK10 4TJ.** Telephone 0625 514549, to whom applications should be sent by 31 May 1989. (9139)E

## FELLOWSHIPS continued

### POSTDOCTORAL FELLOWSHIP

NIH Staff Fellow/Senior Staff Fellow position is available at the National Institute on Alcohol Abuse and Alcoholism, for studies of neurotransmitter and hormone receptors and their interactions with cytokines and growth factors. Preference is given to applicants with experience in molecular biology, protein purification and receptor biochemistry/pharmacology. Salary is commensurate with experience. Only U.S. citizens or permanent residents are eligible for this position.

Send CV and name of three references to:

**Dr. George Kunos**  
Laboratory of Physiologic and  
Pharmacologic Studies NIAAA  
12501 Washington Avenue  
Rockville, MD 20852  
Phone: (301) 443-1234



NIAAA IS AN EQUAL OPPORTUNITY EMPLOYER

(NW3647)E

### CLARE HALL CAMBRIDGE

## RESEARCH FELLOWSHIPS ARTS, SOCIAL SCIENCES AND SCIENCES

The Governing Body of Clare Hall proposes to elect two or more non-stipendiary Research Fellows for a period of three years, starting not earlier than October 1989. It is hoped that the fields of study of these Research Fellows shall include both the Arts and Social Sciences and the Sciences (Physical, Biological and Earth Sciences, Mathematics or Technology). There are no restrictions on age, sex or previous standing, except that those who have previously held College Research Fellowships in Oxford or Cambridge are not eligible, and some preference may be given to candidates who are at a fairly early stage of their research career. The closing date for the receipt of applications and references is **30 June 1989**.

Application forms may be obtained (written requests only, with a stamped, addressed envelope) from The College Secretary, Clare Hall, Herschel Road, Cambridge CB3 9AL, UK. (9130)E

### University of Hong Kong Postdoctoral Fellowship in Patch-clamp technique Department of Physiology

Applications are invited from those with experience of patch-clamp technique, for a Cystic Fibrosis Foundation-supported project on the role of ion channels in fluid secretion in the epididymis.

Appointments will commence as soon as possible and is for 2 years. Annual salary is about US\$22,000 with medical benefits provided. A return airfare will also be provided if required. At current rates, income tax will not exceed 15% of gross income.

Send curriculum vitae, statement of research interests, and the names of three referees to: Dr. P.Y.D. Wong, Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong. Tel: (852) 5-8199202, Telex: 71919 CEREB HX, Fax: (852) 5-8582549. Closing date May 31, 1989. (W6085)E

### POSTDOCTORAL FELLOWSHIPS IN MOLECULAR BIOLOGY AT HARVARD MEDICAL SCHOOL

Unexpected openings exist in July 1989 for two individuals holding Ph.D. or M.D. degrees for NIH supported postdoctoral training in molecular biology of ion transport proteins. Strong backgrounds in biochemistry required. Only U.S. citizens or permanent residents are eligible. Send curriculum vitae and three references to:

**Dr. Barry M. Brenner,**  
Director, Renal Division,  
Brigham and Women's  
Hospital,  
75 Francis Street, Boston,  
MA 02115

HMS/BWH are equal opportunity employers.

(NW3661)E

## STUDENTSHIPS continued

### University of Southampton

### Department of Neurophysiology

## Postgraduate Studentships in Neuroscience

We have two Research Council studentships, tenable for three years, commencing October 1989, to work towards a Ph.D degree in the following areas:

1/ a CASE SERC studentship with Roussel Laboratories, Swindon, to study long term changes in synaptic function as models for learning and memory in rats, involving electrophysiological and behavioural techniques;

2/ an MRC studentship available in one of the following: the molecular basis of pathological conditions by the expression of neuronal mRNAs in *Xenopus* oocytes; the subcellular organisation of ionic channels in mammalian neurones and its physiological and pathological consequences; the spread of primary afferent depolarization in an in vitro spinal cord preparation.

Candidates should have, or expect to obtain, a first or upper second class honours degree of M.Sc. in one of the biological science disciplines. Applications, including a complete C.V. and names and addresses to two referees should be sent to: **Prof. G.A. Kerkut, Dept. of Neurophysiology, University of Southampton, Southampton SO9 3TU.** (9126)F

### UNIVERSITY OF LIVERPOOL DEPARTMENT OF BIOCHEMISTRY

## RNIB RESEARCH STUDENTSHIP

Applications are invited from candidates who possess or expect to obtain a first or upper second class degree in Biochemistry or a related subject for a Research Studentship funded by the Royal National Institute for the Blind. The student will carry out research into the molecular biology of the vertebrate retina under the supervision of Professor B. E. H. Maden. The stipend is the same as for MRC studentships; however, in addition to the standard bench allowances there is substantial extra funding for consumable materials.

Interested candidates should send a curriculum vitae and the names of two referees to **Professor Maden, Department of Biochemistry, The University, P.O. Box 147, Liverpool, L69 3BX.**

An Equal Opportunity Employer.

(9080)F

### The Queen's University of Belfast RESEARCH STUDENTSHIPS School of Biology and Biochemistry

A number of research studentships will be available, commencing 1st October 1989, in the following areas:

**Biochemistry:** (peptide, nucleic acid and immuno chemistry).

**Genetic Engineering:** (virology, oncology, microbial and plant molecular biology).

**Cell and Experimental Biology:** (parasitology and plant physiology).

**Environmental and Evolutionary Biology:** (behaviour, ecology, fish biology and marine biology).

Applicants should have or expect to obtain at least a 2.1 degree or equivalent qualification in an appropriate biological science subject and be highly motivated for a research career. Applications should be submitted to **The Director, Professor S.J. Martin, School of Biology and Biochemistry, Queen's University, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland,** and include a curriculum vitae, the names of two referees and a brief description of their research experience. The studentships are available for residents in EEC countries and will carry a stipend of £3,000 pa plus payment of fees. Closing date 30th May, but late applications may be considered. (9116)F

### AFRC INSTITUTE FOR GRASSLAND AND ANIMAL PRODUCTION WELSH PLANT BREEDING STATION ABERYSTWYTH RESEARCH STUDENTSHIP

Applications are invited for an AFRC funded three year PhD studentship to commence in October 1989 for work on genetic and molecular regulation of leaf senescence in mutants of *Arabidopsis thaliana*. The appointee will acquire a range of genetic, biochemical, immunological and

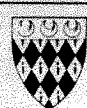
molecular biological skills.

Applicants should hold or expect to obtain a first or upper second class honours degree in Biochemistry, Genetics, Physiology, Cell/Molecular Biology or a related subject. The successful candidate will be registered for a PhD at the University of Wales, Aberystwyth.

Further details and application forms are available from the Personnel Officer, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB. Closing date 31 May 1989.

(9138)F





ROYAL HOLLOWAY AND BEDFORD  
**NEW COLLEGE**  
University of London

DEPARTMENT OF GEOLOGY

## RESEARCH STUDENTSHIP IN STABLE ISOTOPE GEOCHEMISTRY

A 3 year studentship, supported by VG Isogas Ltd, is available for research into the stable isotope geochemistry of fluid processes in the mantle and lower crust. Fluid inclusion geochemistry will be investigated using state-of-the-art gas extraction and mass spectrometric techniques.

The student will be based in the new stable isotope laboratory at RHBNC and will also have opportunities to utilise research and developmental facilities at VG Isogas Ltd. Attendance at international conferences will be encouraged.

The project will be jointly supervised by Dr D P Matthey (RHBNC) and Dr R A Exley (VG Isogas).

Applications, including a brief CV and names of two referees, should be sent to: **Dr D P Matthey, Department of Geology, RHBNC, University of London, Egham Hill, Egham, Surrey. TW20 0EX.** To obtain further information please telephone Egham (0784) 34455 ext. 3587.

(9070)F

DEPARTMENT OF MICROBIOLOGY  
UNIVERSITY OF SOUTHAMPTON

## MRC RESEARCH STUDENTSHIPS

Applications are invited from students who expect to obtain a B.Sc. I or II (i) in Biochemistry, Genetics, Microbiology or related subject for four MRC Research Studentships tenable from 1st October, 1989.

The main interests of the Department are in understanding the molecular basis of microbial pathogenicity. The Department has major funding for research on bacteria (chlamydia, meningococci, gonococci) and viruses (respiratory syncytial, varicella-zoster). Our research programmes are directed towards the development of both vaccines and improved diagnostic reagents for these important infectious agents.

The Department is well equipped and has excellent facilities for advanced techniques in molecular genetics (including automated oligonucleotide synthesis) and immunobiology. The research studentship will involve the integrated application of recombinant DNA technology, protein chemistry, immunology and cell biology. These projects offer an ideal opportunity to gain highly relevant expertise in a spectrum of modern disciplines and comprehensive training will be given.

Further information can be obtained from **Dr I N Clarke (Tel. 0703 777222 Ext. 3886), Department of Microbiology (University), South Laboratory and Pathology Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY** to whom applications in the form of a full CV and the names of two academic referees should be sent.

(9078)F

UNIVERSITY COLLEGE OF SWANSEA  
School of Biological Sciences

Applications are invited for four NERC funded

## Ph.D. STUDENTSHIPS

available in the Biomedical and Physiological Research Group. Studentships 1, 2 and 3 are to commence in October 1989, 4 is available immediately.

1. Molecular analysis of mutation frequencies in *Mytilus* larvae. (Professor J M Parry and Dr D H Jones).
2. The monitoring of chronic exposure to genotoxic chemicals (CASE award with the Severn-Trent Water Authority). (Professor J M Parry, Dr A F Rowley and Dr R Tye).
3. Mitochondrial DNA polymorphism and fitness characters in marine bivalves. (Dr D O F Skibinski).
4. Natural selection of nuclear and mitochondrial DNA in hybrid *Mytilus* populations. (Dr D O F Skibinski).

The projects will be carried out in a well funded department and students will be provided with the opportunity of training in a wide variety of techniques. Previous experience of techniques of molecular biology are not required but applicants should have enthusiasm for working on interesting scientific problems. Opportunities for postgraduate research are also available in other areas of biology.

Please send requests for further information or letters of application with c.v. to **Dr D O F Skibinski, School of Biological Sciences, University College of Swansea, Swansea SA2 8PP, U.K.** as soon as possible.

(9124)F

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ROYAL HOLLOWAY AND BEDFORD  
**NEW COLLEGE**  
University of London

DEPARTMENT OF BIOCHEMISTRY

in collaboration with

**AFRC INSTITUTE OF ARABLE CROPS RESEARCH**  
Rothamsted Experimental Station

## SERC CASE PhD STUDENTSHIP

Applications are invited from graduates in the biological sciences for a three year studentship for working on the isolation and characterisation of plant cytochrome P450 enzymes involved in herbicide detoxification in resistant plant species. The student will be registered at Royal Holloway and Bedford New College, but will spend equal time at both institutions. The project will be under the supervision of Prof J R Bowyer (RHBNC) and Dr D L Hallahan (RES).

The student will gain experience in plant tissue culture, membrane protein purification (primarily using IPLC), the use of radiochemicals in metabolism studies, spectroscopic analysis and monoclonal antibody technology.

Applications, with the names and addresses of two referees, should be submitted by June 14th to **Professor J R Bowyer, Department of Biochemistry, RHBNC, Egham Hill, Egham, Surrey. TW20 0EX.**

Informal enquiries may be made by telephoning Prof Bowyer (0784 34455 ext. 3803) or Dr Hallahan (05827 63133 ext. 2264). (9117)F

INSTITUTE OF PLANT SCIENCE RESEARCH,  
CAMBRIDGE LABORATORY

## AFRC POSTGRADUATE RESEARCH STUDENTSHIP

Applicants are invited for a postgraduate studentship, which is tenable from 1st October 1989, to work with Dr. George Coupland. The objective of the project is to vary the transcription level of the transposase gene of the maize transposon *Ac* in order to investigate control of transposition frequency in *Arabidopsis*. It is hoped that this analysis will lead to an efficient transposon-tagging system in *Arabidopsis*.

Candidates should have or expect to gain a 1st or 2(i) Honours degree. Further information available on request. Applications with CV and the names of two referees to **Mrs L. Cliff, IPSR Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB**, quoting reference MG/PGF/2 by 15th May. (9137)F

UNIVERSITY OF NEWCASTLE UPON TYNE  
Division of Pathology

## MRC Studentship

Applications are invited for a Ph.D. Studentship to work in one of the following areas: cancer research, human genetics, virology.

Applicants should possess or expect to obtain at least an upper second class Honours degree in biochemistry, biology, genetics, microbiology or a related subject.

Applications together with the names of two referees or enquiries for further particulars should be addressed to: **Professor C H W Horne, Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP (Tel: 091 2328511 ext. 7144).** (9119)F

University of York

## Research Studentship

'UNSELFISH' B-CHROMOSOMES IN POPULATIONS OF WILD CHIVES

A NERC-funded studentship is available in the Department of Biology for studies on 'unselfish' B-Chromosomes in populations of wild chives (*Allium schoenoprasum*), specifically to investigate the nature of their advantage for the survival of individuals in natural and experimental populations. The studentship will be tenable from October 1989. Applicants should have or expect to obtain at least an upper second class honours degree in an appropriate biological subject.

Applications, including C.V. and the names of two academic referees, should be sent to **Dr. S. M. Bougourd, Department of Biology, University of York, York YO1 5DD.** Further details are available on request.

(9120)F

**UNIVERSITY OF LEICESTER,  
DEPARTMENT OF BIOCHEMISTRY**

**POSTGRADUATE STUDENTSHIPS**

Applications are invited for the following:

**1. SERC/CASE STUDENTSHIP.** "Energy Coupling in DNA Gyrase" — Dr. A. Maxwell. To study the coupling between the free energy of ATP hydrolysis and the introduction of supercoils into DNA by gyrase. The project will involve both molecular biology and enzymology (Supported by Glaxo.)

**2. SERC/CASE STUDENTSHIP.** "Structure and Mechanism of Serine Acetyltransferase" — Prof. W.V. Shaw and Dr. D.B. Wigley. To investigate the relationship between the structure and mechanism of this and other acyl transferases. Techniques will include crystallography, enzymology and site-directed mutagenesis. (Supported by Smith, Kline and French.)

**3. SERC/BIOTECHNOLOGY "EARMARKED" STUDENTSHIP.** "NMR and Mutagenesis Studies of Dihydrofolate Reductase" — Prof. G.C.K. Roberts. To study the role of individual amino-acid residues in the specificity of drug binding to this enzyme; the work will involve site-directed mutagenesis, enzymology and/or nmr spectroscopy.

**4. SERC/BIOTECHNOLOGY "EARMARKED" STUDENTSHIP.** "NMR and Mutagenesis Studies of the *E.coli* Trp Repressor" — Prof. G.C.K. Roberts and Prof. W.J. Brammar. To investigate the mechanism by which this protein recognises specific DNA sequences, using molecular biological and spectroscopic methods.

**5. SERC and MRC** quota studentships in the areas of Biomolecular Structure and Dynamics, Bioconversions and Biotechnology, and Eukaryotic Molecular Biology. Details of individual projects are available on request from Dr. R.A. Cooper.

Further information may be obtained by contacting the persons named above (tel: (0533) 523470). Applications should include a c.v. and the names of two academics referees and should be submitted to **Dr. R.A. Cooper, Department of Biochemistry, University of Leicester, Leicester LE1 7RH.** (9121)F

**IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY  
AND MEDICINE**

**DEPARTMENT OF BIOCHEMISTRY and CENTRE  
FOR BIOTECHNOLOGY**

**POSTGRADUATE STUDENTSHIPS**

<b>Prof HF Bradford</b>	Synaptosomes and neurotransmitters.
<b>Dr A Cass</b>	NMR and biosensors.
<b>Dr S Cotterill</b>	DNA replication in <i>Drosophila melanogaster</i> .
<b>Dr D Cutler</b>	Intracellular traffic of membrane proteins.
<b>Dr A Dell</b>	Glycobiology and mass spectrophotometry.
<b>Dr JO Dolly</b>	Neurotransmission and K <sup>+</sup> channels.
<b>Prof B Hartley</b>	Protein engineering.
<b>Prof CR Hopkins</b>	Membrane traffic and cell surface receptors.
<b>Dr D Leak</b>	Biotransformations and microbial physiology.
<b>Dr C Lichtenstein</b>	Genetic engineering of plants and bacteria.
<b>Dr P Little</b>	Genetic map of human chromosome 11.
<b>Dr P Mantle</b>	Microbial products; mycotoxins and neurotoxins.
<b>Prof H Morris</b>	Metabolism and measurement of leukotrienes.
<b>Dr K O'Hare</b>	Molecular genetics of <i>Drosophila</i> .
<b>Dr M Selkirk</b>	Molecular immunology of parasite antigens.
<b>Dr DF Smith</b>	Molecular parasitology of leishmaniasis.
<b>Dr J Tippins</b>	Calcitonin gene-related peptide and vasodilation.
<b>Dr G Wilkin</b>	Astroglial/oligodendroglial cells in the CNS.

Applications with a full CV and details of 2 referees, should be sent as soon as possible to **Dr AG Dickerson, Biochemistry Department, Imperial College, London SW7 2AZ.** (9103)F

**Ludwig Institute for Cancer Research  
St Mary's Branch  
PhD Studentship**

Applications are invited for a 3 year PhD studentship in the field of oncogene research. Our laboratory applies a wide range of molecular and biological techniques to study the molecular aspects of oncogenesis and differentiation with emphasis on function and regulation of expression of the *Mos* proto oncogene. Applicants holding or expecting a good degree in the biological sciences should send a curriculum vitae and the names of two references to **Dr. Friedrich Propst, Ludwig Institute for Cancer Research, St Mary's Medical School, Norfolk Place, London W2 1PG. Tel: 01-724 5522.** (9131)F



*University  
of Reading*

**Appointments**

**SCHOOL OF ANIMAL & MICROBIAL SCIENCES  
STUDENTSHIP**

available to study use of avian primordial germ cells in production of transgenic birds. Applicants should have a good knowledge of experimental embryology or molecular biology. Value £4000/y plus fees for higher degree registration.

**Send c.v. plus names of 2 referees to Professor K. Simkiss, Dept of Pure & Applied Zoology, University of Reading, Whiteknights, P O Box 228, Reading RG6 2AJ.** (9085)F

**AFRC INSTITUTE OF  
ARABLE CROPS  
RESEARCH**

**AFRC RESEARCH STUDENTSHIPS**

Applications are invited for the following AFRC Studentships to commence in October 1989:

1. RFLP analysis, including DNA fingerprinting, of insects resistant and susceptible to insecticides, with Dr A Devonshire and Dr I Denholm, Insecticides Department, IACR-RES.
2. The role of glutamine synthetase in determining the efficiency of symbiotic N-fixation, with Dr B Forde, Biochemistry Department, IACR-RES.
3. Gibberellin receptors and signal transduction in aleurone protoplasts of *Avena fatua* L., with Dr R Hooley, University of Bristol, Department of Agricultural Sciences, IACR-RES.

Projects 1 and 2 are based at IACR-Rothamsted Experimental Station, Harpenden, and project 3 at IACR-Long Ashton Research Station, Bristol.

Applicants should have, or expect to gain, a first or upper second class honours degree. The studentships are tenable for three years and the successful candidates will be registered for an M.Phil or a Ph.D with a relevant University.

Applications, indicating the project selected and giving names and addresses of two referees, should be sent to the Institute Secretary, IACR, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, quoting Ref: 795, by 19 May. Informal telephone enquiries can be made to the supervisors named on 0272 392181 for Long Ashton or 05827 63133 for Rothamsted. (9136)F

**UNIVERSITY OF SHEFFIELD  
DEPARTMENT OF MOLECULAR  
BIOLOGY AND BIOTECHNOLOGY**

**STUDENTSHIP IN  
ANTIBODY  
ENGINEERING**

An SERC-funded CASE studentship is available for a suitably qualified graduate to work on a collaborative project of antibody engineering between Dr Dennis Burton and Celltech. The project will use techniques of molecular biology to design antibodies with novel properties.

The studentship is available immediately but start date is negotiable. Applications with a c.v. and names of two referees and informal enquiries should be directed to **Dr Dennis Burton, Department of Molecular Biology and Biotechnology, The University, Sheffield S10 2TN** (tel: 0742 768555 ext 4323). (9084)F

**University of St Andrews  
DEPARTMENT OF BIOLOGY  
& PRECLINICAL MEDICINE  
Studentship in  
Molecular Electronics**

Applications are invited for an SERC Studentship in Molecular Electronics to work on a project to investigate "Properties of small artificial circuits of identifier neurones". Candidates should have or expect to obtain a First or Upper Second Class Degree in a Biological Science. Letters of application enclosing a full Curriculum vitae, which includes the names and addresses of two academic referees, should be sent to **Professor G.A. Cottrell, Department of Biology & Pre-clinical Medicine, Bute Medical Buildings, University of St Andrews, St Andrews, Fife, KY16 9TS**, from whom further particulars are also available. (9097)F

## SEMINARS & SYMPOSIA

### THE THIRD CAMBRIDGE MINI-SYMPOSIUM (a series devoted to the exploration of the molecular and cellular aspects of drug actions).

**JULY 7TH-8TH 1989**

#### "RECEPTOR STRUCTURE AND FUNCTION"

Organised jointly by the Department of Pharmacology, University of Cambridge, the MRC Neurobiology Unit and the Parke-Davis Research Unit. To be held at Churchill College, Cambridge.

In the 1989 Symposium we intend to explore the relationship between the structure and function of both membrane-bound and non membrane-bound proteins. The discussions are intended to range from X-ray and nmr data on receptor structure, enzyme and receptor-ligand interactions, through to functional responses involving, for example, linking to channels and regulatory proteins.

The audience and participants are intended to be multidisciplinary comprising of pharmacologists, biochemists and medicinal chemists.

Invited Speakers include: T. Blundell (London), G. Wagner (Michigan), R. Schwyzler (Zurich), A. Fersht (Cambridge), J. Saunders (M.S.D.), P. Goodford (Oxford), P. Dean (Cambridge), A. Walmsley (Leicester), C. Venter (Rockville), N. Unwin (Cambridge).

Attendance is limited to invited participants selected from those applying as outlined below.

Poster Session. Posters are invited from participants. Please indicate your intention to present a poster and a suggested title with your application.

Registration details: The Registration Fee of £25 covers the meeting and accommodation at Churchill College on 7th July, lunches on 7th and 8th July, breakfast on 8th July and the Symposium Dinner on 8th July.

A limited number of students and post-doctoral workers within three years of qualification will be given free registration and accommodation. In exceptional circumstances, a travel bursary may be available. Applicants for these places and bursaries should enclose a letter of support from their Head of Department.

Please send applications by 12th May enclosing a cheque for £25 payable to Churchill College, Cambridge and a brief résumé of your research interests to:

THE SYMPOSIUM SECRETARY  
PARKE DAVIS RESEARCH UNIT  
ADDENBROOKES HOSPITAL SITE  
HILLS ROAD  
CAMBRIDGE CB2 2QB  
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## COURSES & CONFERENCES

### COLD SPRING HARBOR LABORATORY FALL COURSES



#### Macromolecular Crystallography

**October 11 - 24, 1989**

Alexander McPherson, *University of California, Riverside*

Jim Pflugrath, *Cold Spring Harbor Laboratory*

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensive laboratory computational course will focus on the major techniques used to determine the 3-dimensional structures of macromolecules. It is designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics that will be covered include: protein purification, crystallization, crystal characterization, data collection (film and area detector methods), data reduction, anomalous dispersion, phase determination, molecular replacement and averaging, electron density interpretation, structure refinement, molecular graphics, and molecular dynamics. Participants will learn through extensive hands-on experiments, informal discussions and lectures on current applications of these and related procedures given by outside speakers.

#### Molecular Genetics of Fission Yeast

**October 24 - November 6**

David Beach, *Cold Spring Harbor Laboratory*

Peter Fantes, *University of Edinburgh, Scotland*

Jerry Hyams, *University College, London*

Maureen McLeod, *Cold Spring Harbor Laboratory*

The fission yeast is increasingly being used as a model organism for the study of basic aspects of cell biology. The course to be held at Cold Spring Harbor Laboratory October - November, 1989 will introduce the students to all aspects of fission yeast biology, but with particular emphasis on genetic manipulation (both classical and with recombinant DNA) and the use of the organism for the study of cell biology. It is hoped that this course will be particularly valuable because very few laboratories in the United States have substantial experience with fission yeast, in contrast with Western Europe and Japan.

Tuition, room and board ..... \$1315.

Application Deadline: ..... July 15, 1989

Applications and additional information may be obtained from:

Registrar

Cold Spring Harbor Laboratory

Cold Spring Harbor, New York 11724

Phone: (516) 367-8343

Fax: (516) 367-8845

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## Two forthcoming International Conferences

### ADENOSINE AND ATP

#### Progress in Research and Therapeutic Potential

25th/26th September 1989 — London

In recent years, the discovery of a number of purinoceptor subtypes has prompted an explosion of interest in research on purine nucleosides and nucleotides, e.g. adenosine, ADP, ATP etc. These substances exert potent and selective effects in a variety of tissues, from the nervous system and all types of muscle, to endothelial, epithelial, secretory and immune cells. Intense efforts are currently underway in both academia and industry aimed at developing selective agonists and antagonists for the different subclasses of purinoceptors, and at exploring the therapeutic potential of such compounds. This meeting will review the progress that has been made to date.

### THE PLATELET IN HEALTH AND DISEASE

24th/25th October 1989 — London

This meeting will bring together experts from a variety of backgrounds to discuss the latest evidence for the role that platelets play in both health and disease. Although well established as participants in haemostasis and disorders such as thrombosis and atherosclerosis, there is now a growing recognition that the platelet has both additional physiological and pathophysiological roles. Where possible, the meeting will attempt to identify new avenues for drug development based on current knowledge of platelet function.

For further information about both conferences, please contact:

Renata Duke,

IBC Technical Services Ltd., Bath House (3rd Floor), 56 Holborn Viaduct, London EC1A 2EX

Tel: 01-236 4080 Fax: 01-489 0849 Telex: 888870 IBC G

(9107)C



### UNIVERSITY OF LONDON

Royal Postgraduate Medical School and  
University College London

#### MSc in Neuroendocrine Cell Biology

Applications are invited from graduates in medicine or science for entry into this one-year degree course in the Faculty of Medicine. Students will receive training in aspects of neuroscience and endocrinology and emphasis will be placed on neuroendocrine tissues as an integrated system. Modern investigative techniques, including those currently used in molecular biology, neuropharmacology, immunohistochemistry, electron microscopy, tissue/cell culture, neuroanatomy and receptor studies will be covered. Students will work at both institutions and formal teaching, in the form of tutorials, lectures, practical demonstrations and specialist short courses will be conducted by both host departments (Anatomy and Developmental Biology at University College London; Histochemistry at the Royal Postgraduate Medical School). Each student will carry out an extended research project. Award of the MSc degree will be by written examination and submitted dissertation.

Further information and application form may be obtained from Professor J Polak, Department of Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN or Professor G Burnstock, Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT. Enquiries may be made to the Course Secretary, (tel: 01-387 7050, ext 3335). (9073)C

### UMIST

#### ESSENTIAL TECHNIQUES IN GENE MANIPULATION A PRACTICAL COURSE — 11-16 SEPTEMBER 1989

This is an intensive practical course designed for people with little or no experience of recombinant DNA techniques. The practical work will cover the following topics:

1. PRINCIPLES OF DNA ISOLATION AND HANDLING: Purification of DNA, restriction analysis, agarose gel electrophoresis, Southern transfer, DNA labelling, hybridisation probing.
2. BASIC CLONING TECHNIQUES: Preparation of plasmid and phage DNA, transformation, in vitro packaging, construction of recombinant DNA molecules, recombinant selection with plasmid and phage vectors.
3. DNA SEQUENCING: Preparation of template DNA, chain termination sequencing, computer-aided sequence analysis.

Additional topics will be demonstrated and discussed in seminars: cDNA synthesis and cloning, genomic libraries, site-directed mutagenesis, preparation and uses of oligonucleotides and peptides, separation of large molecules by OFAGE.

The course is sponsored by Amersham International, BioRad and BHD, and will take place in the teaching and research laboratories of the Applied Molecular Biology Group and Manchester Biotechnology Centre. The fee is £475 + £75 for accommodation.

Application forms from Dr T A Brown, Department of Biochemistry and Applied Molecular Biology, UMIST, P O Box 88, Manchester M60 1QD, UK. Telephone enquiries to 061 236 3311 exts. 2139 or 2159. The course will be limited to 25 participants. (9113)C

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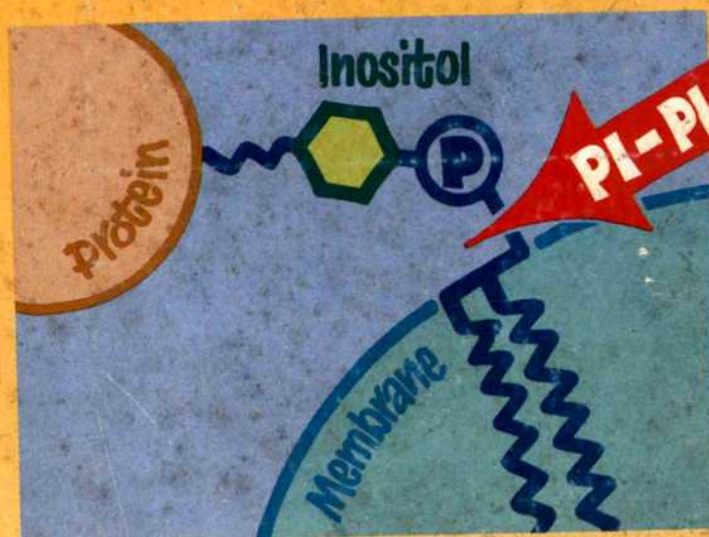
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